Title: TREATMENT OF FRUITS OR VEGETABLES WITH HYPERSENSITIVE RESPONSE ELICITOR TO CONTROL POSTHARVEST DISEASE OR DESICCATION

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TREATMENT OF FRUITS OR VEGETABLES WITH HYPERSENSITIVE RESPONSE ELICITOR TO INHIBIT POSTHARVEST DISEASE OR DESICCATION

This application claims benefit of U.S. Provisional Patent Application Serial No. 60/198,359, filed April 19, 2000, which is hereby incorporated by reference in its entirety.

FIELD OF THE INVENTION

The present invention relates to methods of treating fruits or vegetables to inhibit postharvest diseases and/or desiccation of harvested fruits or vegetables.

BACKGROUND OF THE INVENTION

Postharvest diseases are often extensions of disease occurring in the field or orchard. Brown rot of stone fruits (*Monilinia fructicola* (Wint.) Honey), for example, may cause blossom and twig blighting in the orchard. Infections in the orchard may not be visible at harvest if fruits are not refrigerated. *Colletotrichum gloeosporioiders* (Penz.) Arx may attack blossoms or leaves and young fruit of citrus, avocados, mangos, papayas, and a wide range of other tropical and subtropical species; infections in developing fruit are usually latent, and rot lesions appear only at the onset of fruit ripening. *Pezicula malicorticis* (Jacks.) Nannfld. causes cankers of limbs of apples and pears; infections in developing fruit are latent, and active rotting usually commences only after the fruit has spent several months in storage and proceeds during -1°C storage because the organism is able to grow at very low temperatures. These fungi used as examples are able to penetrate the cuticle and epidermis of the fruit.

Whether capable of being penetrated directly or not, wounds are often the usual means by which the fungus enters fruit. Cuts, punctures, bruises, and abrasions cannot be avoided completely during harvest and handling. If the cuticle and epidermis are broken, spores find nutrients and humidity in fresh wounds ideal for spore germination and colonization. Separation of fruits from the parent plant at harvest creates an unavoidable wound that encourages stem-end rots.

Rots developing at the blossom end usually involve prior colonization of floral parts. For example, *Botrytis* blossom-end rot (*B. cinerea*) sometimes occurs in Bartlett pears after a month or two in storage at -1°C. Initiation of rot in fruit flesh is associated with old styles and stamens retained within the fruit. Floral infections occur in the senescing floral parts at the end of blossoming. Mostly these floral parts are invaded by *Alternaria* spp. and common saprophytic fungi, but *B. cinerea* also is found occasionally. Not all fruits having *B.cinerea*-invaded floral parts rot in storage, but a significant percentage do. By contrast, test fruits remain free from *Botrytis* blossom-end rot if the old floral parts of developing fruits are free from *B. cinerea*. Rotting of fruits in storage is greatly reduced by a single orchard spray with a fungicide at the end of blossoming.

Contact infection, by which mycelia grow from a rotting fruit to contact and penetrate nearby fruit, is an especially serious aspect of some very common postharvest pathogens. The ever-enlarging "nest" of rotting fruit tied together by fungus mycelia will involve all fruit in a container, if given sufficient time.

Disease or threat of disease dictates in large measure the manner in which perishable fruits are handled. In recent decades, fruits have been shipped to increasingly greater distances from points of production. Exploitation of these distant markets, however, may offer large economic benefits only if the life of the commodity is stretched to its limit. Diseases and disorders ordinarily manageable during handling and transcontinental transit and marketing may be excessive when transoceanic marine transport of longer duration is involved. Similarly, the extension of marketing periods by storing fruits until they near the end of their physiological life may cause additional disease problems. Losses are especially serious if they occur in market areas, because the costs of sorting, packaging, cooling, storage, and transportation, which may greatly exceed production costs, have already been incurred. Of even greater long-term importance may be an impaired reputation leading to reduced future sales.

Postharvest diseases of fruit cause 15 to 25% losses yearly in the fruit industry worldwide and much of this is due to rot caused by microorganisms. Fungicides, which have been the primary means of controlling postharvest diseases, have come under scrutiny as posing potential oncogenic risks when applied to

processed foods. Thus, research efforts have been intensified to develop biological control procedures for postharvest diseases of fruits and vegetables that pose less risk to human health and the environment.

Considerable attention has been placed on assessing the use of antagonistic microorganisms as a viable alternative to the use of synthetic fungicides. Two basic approaches are available for using antagonistic microorganisms to control postharvest diseases. Naturally occurring antagonists that already exist on fruit and vegetable surfaces have been shown to control several rot pathogens on diverse commodities. Alternatively, artificially introduced antagonists have been shown to be effective in biologically controlling postharvest pathogens.

Since 1983, an explosion of research has occurred in the area of biological control of postharvest diseases by artificially introduced antagonists, mostly on fruit diseases (Janisiewicz, "Biological Control of Diseases of Fruit," In Biocontrol of Plant Diseases II, Mukergie et al. (ed.), CRC Press, Boca Raton, pp. 153-165 (1988) and Wilson et al., "Potential for Biological Control of Postharvest Plant Diseases," Plant Disease 69:375-378 (1985)). For example, rot on apples was controlled with yeast (Wisniewski et al., "Biological Control of Postharvest Diseases of Fruit: Inhibition of *Botrytis* Rot on Apples by an Antagonistic Yeast," Proc. Electron Microsc. Soc. Am. 46:290-91 (1988)), while brown rot in apricots was controlled with *Bacillus subtilis* (Pusey et al., "Postharvest Biological Control of Stone Fruit Brown Rot by *Bacillus subtilis*," Plant Dis. 68:753-56 (1984)). Mold incidence was reduced from 35% to 8% in lemon peel by a species of *Trichoderma* (De Matos, "Chemical and Microbiological Factors Influencing the Infection of Lemons by *Geotrichum candidum* and *Penicillium digitatum*," Ph.D. dissertation, University of California, Riverside, 106 pp. (1983)). Biocontrol of citrus rot pathogens was demonstrated with *Bacillus subtilis* (Singh et al., "*Bacillus subtilis* as a Control Agent Against Fungal Pathogens of Citrus Fruit," Trans. Br. Mycol. Soc. 83:487-90 (1984)). Such antagonists have various modes of action: antibiosis or competition for nutrients and space or both, induction of resistance in the host tissue, and direct interaction with the pathogen (Wilson et al., "Biological Control of Postharvest Diseases of Fruits and Vegetables: An Emerging Technology," Annu. Rev. Phytopathol. 27:425-441 (1989)).

While treatment with antagonistic bacterial or fungal species may be, at least to some extent, effective in controlling postharvest diseases, there are a number of factors which must be considered before this approach is used in commercial applications. First, the antagonists must be grown and maintained for use in treatments. This may result in significant expense and regulatory burdens depending on when and how frequently such antagonists would be applied. Also, it is questionable whether growers would want to maintain bioreactors for growing and propagating particular antagonist strains. Second, the efficacy of those antagonists may depend on storage conditions during shipment of harvested fruit. Some antagonists may not be able to tolerate variations in conditions during shipment, thereby allowing the pathogens to overcome any inhibitory effects of the antagonists. Given the above problems, it is not surprising that few of the antagonists reported to control plant pathogens have been successfully transferred from the laboratory into the field or postharvest environment.

Thus, there still exists a need to provide an effective, commercially viable method for treating fruits and vegetables to control postharvest diseases which avoids entirely or otherwise significantly reduces the need for fungicide treatments. In particular, it would be desirable to provide an effective, practicable treatment which presents little or no harm to humans or the environment.

The present invention is directed to overcoming these and other deficiencies in the art.

SUMMARY OF THE INVENTION

The present invention relates to a method of inhibiting postharvest disease or desiccation in a fruit or vegetable. This method is carried out treating a fruit or vegetable with a hypersensitive response elicitor protein or polypeptide under conditions effective to inhibit postharvest disease or desiccation.

A further aspect of the present invention relates to another method of inhibiting postharvest disease or desiccation in a fruit or vegetable. This method is carried out by providing a transgenic plant or plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and growing the transgenic plant or transgenic plant produced from the transgenic plant

seed under conditions effective to inhibit a postharvest disease or desiccation in a fruit or vegetable harvested from the transgenic plant.

Another aspect of the present invention relates to a DNA construct that includes a DNA molecule encoding a hypersensitive response elicitor protein or polypeptide, a plant-expressible promoter operably coupled 5' to the DNA molecule, the promoter being effective to transcribe the DNA molecule in fruit or vegetable tissue, and a 3' regulatory region operably coupled to the DNA molecule, wherein expression of the DNA molecule in fruit or vegetable tissue imparts to a fruit or vegetable resistance against postharvest disease or desiccation. Also disclosed are expression systems, host cells, and transgenic plants which contain a heterologous DNA construct of the present invention.

By the present invention, the hypersensitive response elicitor protein or polypeptide can be used to inhibit or otherwise control postharvest diseases (i.e., caused by pathogens) in fruits or vegetables. Likewise, such treatment can also inhibit postharvest desiccation of treated fruits or vegetables. In achieving these objectives, the present invention enables produce growers, warehouse packers, shippers, and suppliers to process, handle, and store fruits and vegetables with reduced losses caused by postharvest disease and desiccation. As a result, the cost of bringing fruits and vegetables from the field to the consumer can be reduced. Importantly, the quality of the treated fruits or vegetables is improved.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a method of inhibiting postharvest disease or desiccation in a fruit or vegetable. This method is carried out treating a fruit or vegetable with a hypersensitive response elicitor protein or polypeptide under conditions effective to inhibit postharvest disease or desiccation.

A further aspect of the present invention relates to another method of inhibiting postharvest disease or desiccation in a fruit or vegetable. This method is carried out by providing a transgenic plant or plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and growing the transgenic plant or transgenic plant produced from the transgenic plant

seed under conditions effective to inhibit a postharvest disease or desiccation in a fruit or vegetable harvested from the transgenic plant.

For use in accordance with these methods, suitable hypersensitive response elicitor proteins or polypeptides are those derived from a wide variety of bacterial and fungal pathogens, preferably bacterial pathogens.

Exemplary hypersensitive response elicitor proteins and polypeptides from bacterial sources include, without limitation, the hypersensitive response elicitors derived from *Erwinia* species (e.g., *Erwinia amylovora, Erwinia chrysanthemi, Erwinia stewartii, Erwinia carotovora*, etc.), *Pseudomonas* species (e.g., *Pseudomonas syringae, Pseudomonas solanacearum,* etc.), and *Xanthomonas* species (e.g., *Xanthomonas campestris*). In addition to hypersensitive response elicitors from these Gram-negative bacteria, it is possible to use elicitors derived from Gram-positive bacteria. One example is the hypersensitive response elicitor derived from *Clavibacter michiganensis* subsp. *sepedonicus.*

Exemplary hypersensitive response elicitor proteins or polypeptides from fungal sources include, without limitation, the hypersensitive response elicitors (i.e., elicitins) from various *Phytophthora* species (e.g., *Phytophthora parasitica, Phytophthora cryptogea*, *Phytophthora cinnamomi*, *Phytophthora capsici*, *Phytophthora megasperma*, *Phytophthora citrophthora*, etc.).

Preferably, the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia chrysanthemi, Erwinia amylovora, Pseudomonas syringae,* or *Pseudomonas solanacearum.*

A hypersensitive response elicitor protein or polypeptide from *Erwinia chrysanthemi* has an amino acid sequence corresponding to SEQ. ID. No. 1 as follows:

```
Met Gln Ile Thr Ile Lys Ala His Ile Gly Gly Asp Leu Gly Val Ser
1               5                   10                  15

Gly Leu Gly Ala Gln Gly Leu Lys Gly Leu Asn Ser Ala Ala Ser Ser
            20                  25                  30

Leu Gly Ser Ser Val Asp Lys Leu Ser Ser Thr Ile Asp Lys Leu Thr
        35                  40                  45

Ser Ala Leu Thr Ser Met Met Phe Gly Gly Ala Leu Ala Gln Gly Leu
    50                  55                  60

Gly Ala Ser Ser Lys Gly Leu Gly Met Ser Asn Gln Leu Gly Gln Ser
65                  70                  75                  80
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Phe Gly Asn Gly Ala Gln Gly Ala Ser Asn Leu Leu Ser Val Pro Lys
                85                  90                  95

Ser Gly Gly Asp Ala Leu Ser Lys Met Phe Asp Lys Ala Leu Asp Asp
            100                 105                 110

Leu Leu Gly His Asp Thr Val Thr Lys Leu Thr Asn Gln Ser Asn Gln
        115                 120                 125

Leu Ala Asn Ser Met Leu Asn Ala Ser Gln Met Thr Gln Gly Asn Met
    130                 135                 140

Asn Ala Phe Gly Ser Gly Val Asn Asn Ala Leu Ser Ser Ile Leu Gly
145                 150                 155                 160

Asn Gly Leu Gly Gln Ser Met Ser Gly Phe Ser Gln Pro Ser Leu Gly
                165                 170                 175

Ala Gly Gly Leu Gln Gly Leu Ser Gly Ala Gly Ala Phe Asn Gln Leu
            180                 185                 190

Gly Asn Ala Ile Gly Met Gly Val Gly Gln Asn Ala Ala Leu Ser Ala
        195                 200                 205

Leu Ser Asn Val Ser Thr His Val Asp Gly Asn Asn Arg His Phe Val
    210                 215                 220

Asp Lys Glu Asp Arg Gly Met Ala Lys Glu Ile Gly Gln Phe Met Asp
225                 230                 235                 240

Gln Tyr Pro Glu Ile Phe Gly Lys Pro Glu Tyr Gln Lys Asp Gly Trp
                245                 250                 255

Ser Ser Pro Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser Lys
            260                 265                 270

Pro Asp Asp Asp Gly Met Thr Gly Ala Ser Met Asp Lys Phe Arg Gln
        275                 280                 285

Ala Met Gly Met Ile Lys Ser Ala Val Ala Gly Asp Thr Gly Asn Thr
    290                 295                 300

Asn Leu Asn Leu Arg Gly Ala Gly Gly Ala Ser Leu Gly Ile Asp Ala
305                 310                 315                 320

Ala Val Val Gly Asp Lys Ile Ala Asn Met Ser Leu Gly Lys Leu Ala
                325                 330                 335

Asn Ala
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This hypersensitive response elicitor protein or polypeptide has a molecular weight of 34 kDa, is heat stable, has a glycine content of greater than 16%, and contains substantially no cysteine. This *Erwinia chrysanthemi* hypersensitive response elicitor protein or polypeptide is encoded by a DNA molecule having a nucleotide sequence corresponding to SEQ. ID. No. 2 as follows:

```
cgattttacc cgggtgaacg tgctatgacc gacagcatca cggtattcga caccgttacg    60
gcgtttatgg ccgcgatgaa ccggcatcag gcggcgcgct ggtcgccgca atccggcgtc   120
gatctggtat ttcagtttgg ggacaccggg cgtgaactca tgatgcagat tcagccgggg   180
cagcaatatc ccggcatgtt gcgcacgctg ctcgctcgtc gttatcagca ggcggcagag   240
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tgcgatggct gccatctgtg cctgaacggc agcgatgtat tgatcctctg gtggccgctg   300
ccgtcggatc ccggcagtta tccgcaggtg atcgaacgtt tgtttgaact ggcgggaatg   360
acgttgccgt cgctatccat agcaccgacg gcgcgtccgc agacagggaa cggacgcgcc   420
cgatcattaa gataaaggcg gctttttta ttgcaaaacg gtaacggtga ggaaccgttt   480
caccgtcggc gtcactcagt aacaagtatc catcatgatg cctacatcgg gatcggcgtg   540
ggcatccgtt gcagatactt ttgcgaacac ctgacatgaa tgaggaaacg aaattatgca   600
aattacgatc aaagcgcaca tcggcggtga tttgggcgtc tccggtctgg ggctgggtgc   660
tcagggactg aaaggactga attccgcggc ttcatcgctg ggttccagcg tggataaact   720
gagcagcacc atcgataagt tgacctccgc gctgacttcg atgatgtttg gcggcgcgct   780
ggcgcagggg ctgggcgcca gctcgaaggg gctggggatg agcaatcaac tgggccagtc   840
tttcggcaat ggcgcgcagg gtgcgagcaa cctgctatcc gtaccgaaat ccggcggcga   900
tgcgttgtca aaaatgtttg ataaagcgct ggacgatctg ctgggtcatg acaccgtgac   960
caagctgact aaccagagca accaactggc taattcaatg ctgaacgcca gccagatgac 1020
ccagggtaat atgaatgcgt tcggcagcgg tgtgaacaac gcactgtcgt ccattctcgg 1080
caacggtctc ggccagtcga tgagtggctt ctctcagcct tctctggggg caggcggctt 1140
gcagggcctg agcggcgcgg gtgcattcaa ccagttgggt aatgccatcg gcatgggcgt 1200
ggggcagaat gctgcgctga gtgcgttgag taacgtcagc acccacgtag acggtaacaa 1260
ccgccacttt gtagataaag aagatcgcgg catggcgaaa gagatcggcc agtttatgga 1320
tcagtatccg gaaatattcg gtaaaccgga ataccagaaa gatggctgga gttcgccgaa 1380
gacggacgac aaatcctggg ctaaagcgct gagtaaaccg gatgatgacg gtatgaccgg 1440
cgccagcatg gacaaattcc gtcaggcgat gggtatgatc aaaagcgcgg tggcgggtga 1500
taccggcaat accaacctga acctgcgtgg cgcgggcggt gcatcgctgg gtatcgatgc 1560
ggctgtcgtc ggcgataaaa tagccaacat gtcgctgggt aagctggcca acgcctgata 1620
atctgtgctg gcctgataaa gcggaaacga aaaaagagac ggggaagcct gtctcttttc 1680
ttattatgcg gtttatgcgg ttacctggac cggttaatca tcgtcatcga tctggtacaa 1740
acgcacattt tcccgttcat tcgcgtcgtt acgcgccaca atcgcgatgg catcttcctc 1800
gtcgctcaga ttgcgcggct gatggggaac gccgggtgga atatagagaa actcgccggc 1860
cagatggaga cacgtctgcg ataaatctgt gccgtaacgt gtttctatcc gccccttag 1920
cagatagatt gcggtttcgt aatcaacatg gtaatgcggt tccgcctgtg cgccggccgg 1980
gatcaccaca atattcatag aaagctgtct tgcacctacc gtatcgcggg agataccgac 2040
aaaatagggc agtttttgcg tggtatccgt ggggtgttcc ggcctgacaa tcttgagttg 2100
gttcgtcatc atctttctcc atctgggcga cctgatcggt t                     2141
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The above nucleotide and amino acid sequences are disclosed and further described in U.S. Patent No. 5,850,015 to Bauer et al. and U.S. Patent No. 5,776,889 to Wei et al., which are hereby incorporated by reference in their entirety.

A hypersensitive response elicitor protein or polypeptide derived from *Erwinia amylovora* has an amino acid sequence corresponding to SEQ. ID. No. 3 as follows:

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Met Ser Leu Asn Thr Ser Gly Leu Gly Ala Ser Thr Met Gln Ile Ser
1               5                   10                  15

Ile Gly Gly Ala Gly Gly Asn Asn Gly Leu Leu Gly Thr Ser Arg Gln
            20                  25                  30

Asn Ala Gly Leu Gly Gly Asn Ser Ala Leu Gly Leu Gly Gly Gly Asn
        35                  40                  45

Gln Asn Asp Thr Val Asn Gln Leu Ala Gly Leu Leu Thr Gly Met Met
    50                  55                  60

Met Met Met Ser Met Met Gly Gly Gly Gly Leu Met Gly Gly Gly Leu
65                  70                  75                  80

Gly Gly Gly Leu Gly Asn Gly Leu Gly Gly Ser Gly Gly Leu Gly Glu
                85                  90                  95

Gly Leu Ser Asn Ala Leu Asn Asp Met Leu Gly Gly Ser Leu Asn Thr
            100                 105                 110

Leu Gly Ser Lys Gly Gly Asn Asn Thr Thr Ser Thr Thr Asn Ser Pro
        115                 120                 125

Leu Asp Gln Ala Leu Gly Ile Asn Ser Thr Ser Gln Asn Asp Asp Ser
    130                 135                 140

Thr Ser Gly Thr Asp Ser Thr Ser Asp Ser Ser Asp Pro Met Gln Gln
145                 150                 155                 160

Leu Leu Lys Met Phe Ser Glu Ile Met Gln Ser Leu Phe Gly Asp Gly
                165                 170                 175

Gln Asp Gly Thr Gln Gly Ser Ser Ser Gly Gly Lys Gln Pro Thr Glu
            180                 185                 190

Gly Glu Gln Asn Ala Tyr Lys Lys Gly Val Thr Asp Ala Leu Ser Gly
        195                 200                 205

Leu Met Gly Asn Gly Leu Ser Gln Leu Leu Gly Asn Gly Gly Leu Gly
    210                 215                 220

Gly Gly Gln Gly Gly Asn Ala Gly Thr Gly Leu Asp Gly Ser Ser Leu
225                 230                 235                 240

Gly Gly Lys Gly Leu Gln Asn Leu Ser Gly Pro Val Asp Tyr Gln Gln
                245                 250                 255

Leu Gly Asn Ala Val Gly Thr Gly Ile Gly Met Lys Ala Gly Ile Gln
            260                 265                 270

Ala Leu Asn Asp Ile Gly Thr His Arg His Ser Ser Thr Arg Ser Phe
        275                 280                 285

Val Asn Lys Gly Asp Arg Ala Met Ala Lys Glu Ile Gly Gln Phe Met
    290                 295                 300

Asp Gln Tyr Pro Glu Val Phe Gly Lys Pro Gln Tyr Gln Lys Gly Pro
305                 310                 315                 320

Gly Gln Glu Val Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser
                325                 330                 335
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Lys Pro Asp Asp Asp Gly Met Thr Pro Ala Ser Met Glu Gln Phe Asn
            340                 345                 350

Lys Ala Lys Gly Met Ile Lys Arg Pro Met Ala Gly Asp Thr Gly Asn
        355                 360                 365

Gly Asn Leu Gln Ala Arg Gly Ala Gly Gly Ser Ser Leu Gly Ile Asp
    370                 375                 380

Ala Met Met Ala Gly Asp Ala Ile Asn Asn Met Ala Leu Gly Lys Leu
385                 390                 395                 400

Gly Ala Ala
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This hypersensitive response elicitor protein or polypeptide has a molecular weight of about 39 kDa, has a pI of approximately 4.3, and is heat stable at 100°C for at least 10 minutes. This hypersensitive response elicitor protein or polypeptide has substantially no cysteine. The hypersensitive response elicitor protein or polypeptide derived from *Erwinia amylovora* is more fully described in Wei, Z-M., et al., "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992), which is hereby incorporated by reference in its entirety. The DNA molecule encoding this hypersensitive response elicitor protein or polypeptide has a nucleotide sequence corresponding to SEQ. ID. No. 4 as follows:

```
aagcttcggc atggcacgtt tgaccgttgg gtcggcaggg tacgtttgaa ttattcataa     60
gaggaatacg ttatgagtct gaatacaagt gggctgggag cgtcaacgat gcaaatttct    120
atcggcggtg cgggcggaaa taacgggttg ctgggtacca gtcgccagaa tgctgggttg    180
ggtggcaatt ctgcactggg gctgggcggc ggtaatcaaa atgataccgt caatcagctg    240
gctggcttac tcaccggcat gatgatgatg atgagcatga tgggcggtgg tgggctgatg    300
ggcggtggct taggcggtgg cttaggtaat ggcttgggtg gctcaggtgg cctgggcgaa    360
ggactgtcga acgcgctgaa cgatatgtta ggcggttcgc tgaacacgct gggctcgaaa    420
ggcggcaaca ataccacttc aacaacaaat tccccgctgg accaggcgct gggtattaac    480
tcaacgtccc aaaacgacga ttccacctcc ggcacagatt ccacctcaga ctccagcgac    540
ccgatgcagc agctgctgaa gatgttcagc gagataatgc aaagcctgtt tggtgatggg    600
caagatggca cccagggcag ttcctctggg ggcaagcagc cgaccgaagg cgagcagaac    660
gcctataaaa aaggagtcac tgatgcgctg tcgggcctga tgggtaatgg tctgagccag    720
ctccttggca acgggggact gggaggtggt cagggcggta atgctggcac gggtcttgac    780
ggttcgtcgc tgggcggcaa agggctgcaa aacctgagcg ggccggtgga ctaccagcag    840
ttaggtaacg ccgtgggtac cggtatcggt atgaaagcgg gcattcaggc gctgaatgat    900
atcggtacgc acaggcacag ttcaacccgt tctttcgtca ataaaggcga tcgggcgatg    960
gcgaaggaaa tcggtcagtt catggaccag tatcctgagg tgtttggcaa gccgcagtac   1020
cagaaaggcc cgggtcagga ggtgaaaacc gatgacaaat catgggcaaa agcactgagc   1080
aagccagatg acgacggaat gacaccagcc agtatggagc agttcaacaa agccaagggc   1140
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atgatcaaaa ggcccatggc gggtgatacc ggcaacggca acctgcaggc acgcggtgcc  1200
ggtggttctt cgctgggtat tgatgccatg atggccggtg atgccattaa caatatggca  1260
cttggcaagc tgggcgcggc ttaagctt                                      1288
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The above nucleotide and amino acid sequences are disclosed are further described in U.S. Patent No. 5,849,868 to Beer et al. and U.S. Patent No. 5,776,889 to Wei et al., which are hereby incorporated by reference in their entirety.

Another hypersensitive response elicitor protein or polypeptide derived from *Erwinia amylovora* has an amino acid sequence corresponding to SEQ. ID. No. 5 as follows:

```
Met Ser Ile Leu Thr Leu Asn Asn Asn Thr Ser Ser Ser Pro Gly Leu
1               5                   10                  15
Phe Gln Ser Gly Gly Asp Asn Gly Leu Gly Gly His Asn Ala Asn Ser
            20                  25                  30
Ala Leu Gly Gln Gln Pro Ile Asp Arg Gln Thr Ile Glu Gln Met Ala
        35                  40                  45
Gln Leu Leu Ala Glu Leu Leu Lys Ser Leu Leu Ser Pro Gln Ser Gly
    50                  55                  60
Asn Ala Ala Thr Gly Ala Gly Gly Asn Asp Gln Thr Thr Gly Val Gly
65                  70                  75                  80
Asn Ala Gly Gly Leu Asn Gly Arg Lys Gly Thr Ala Gly Thr Thr Pro
                85                  90                  95
Gln Ser Asp Ser Gln Asn Met Leu Ser Glu Met Gly Asn Asn Gly Leu
            100                 105                 110
Asp Gln Ala Ile Thr Pro Asp Gly Gln Gly Gly Gly Gln Ile Gly Asp
        115                 120                 125
Asn Pro Leu Leu Lys Ala Met Leu Lys Leu Ile Ala Arg Met Met Asp
    130                 135                 140
Gly Gln Ser Asp Gln Phe Gly Gln Pro Gly Thr Gly Asn Asn Ser Ala
145                 150                 155                 160
Ser Ser Gly Thr Ser Ser Ser Gly Gly Ser Pro Phe Asn Asp Leu Ser
                165                 170                 175
Gly Gly Lys Ala Pro Ser Gly Asn Ser Pro Ser Gly Asn Tyr Ser Pro
            180                 185                 190
Val Ser Thr Phe Ser Pro Pro Ser Thr Pro Thr Ser Pro Thr Ser Pro
        195                 200                 205
Leu Asp Phe Pro Ser Ser Pro Thr Lys Ala Ala Gly Gly Ser Thr Pro
    210                 215                 220
Val Thr Asp His Pro Asp Pro Val Gly Ser Ala Gly Ile Gly Ala Gly
225                 230                 235                 240
Asn Ser Val Ala Phe Thr Ser Ala Gly Ala Asn Gln Thr Val Leu His
                245                 250                 255
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Asp Thr Ile Thr Val Lys Ala Gly Gln Val Phe Asp Gly Lys Gly Gln
            260                 265                 270

Thr Phe Thr Ala Gly Ser Glu Leu Gly Asp Gly Gly Gln Ser Glu Asn
        275                 280                 285

Gln Lys Pro Leu Phe Ile Leu Glu Asp Gly Ala Ser Leu Lys Asn Val
    290                 295                 300

Thr Met Gly Asp Asp Gly Ala Asp Gly Ile His Leu Tyr Gly Asp Ala
305                 310                 315                     320

Lys Ile Asp Asn Leu His Val Thr Asn Val Gly Glu Asp Ala Ile Thr
                325                 330                 335

Val Lys Pro Asn Ser Ala Gly Lys Lys Ser His Val Glu Ile Thr Asn
            340                 345                 350

Ser Ser Phe Glu His Ala Ser Asp Lys Ile Leu Gln Leu Asn Ala Asp
        355                 360                 365

Thr Asn Leu Ser Val Asp Asn Val Lys Ala Lys Asp Phe Gly Thr Phe
    370                 375                 380

Val Arg Thr Asn Gly Gly Gln Gln Gly Asn Trp Asp Leu Asn Leu Ser
385                 390                 395                     400

His Ile Ser Ala Glu Asp Gly Lys Phe Ser Phe Val Lys Ser Asp Ser
                405                 410                 415

Glu Gly Leu Asn Val Asn Thr Ser Asp Ile Ser Leu Gly Asp Val Glu
            420                 425                 430

Asn His Tyr Lys Val Pro Met Ser Ala Asn Leu Lys Val Ala Glu
        435                 440                 445
```

This protein or polypeptide is acidic, rich in glycine and serine, and lacks cysteine. It is also heat stable, protease sensitive, and suppressed by inhibitors of plant metabolism. The protein or polypeptide of the present invention has a predicted molecular size of ca. 4.5 kDa. The DNA molecule encoding this hypersensitive response elicitor protein or polypeptide has a nucleotide sequence corresponding to SEQ. ID. No. 6 as follows:

```
atgtcaattc ttacgcttaa caacaatacc tcgtcctcgc cgggtctgtt ccagtccggg    60
ggggacaacg ggcttggtgg tcataatgca aattctgcgt tggggcaaca acccatcgat   120
cggcaaacca ttgagcaaat ggctcaatta ttggcggaac tgttaaagtc actgctatcg   180
ccacaatcag gtaatgcggc aaccggagcc ggtggcaatg accagactac aggagttggt   240
aacgctggcg gcctgaacgg acgaaaaggc acagcaggaa ccactccgca gtctgacagt   300
cagaacatgc tgagtgagat gggcaacaac gggctggatc aggccatcac gcccgatggc   360
cagggcggcg ggcagatcgg cgataatcct ttactgaaag ccatgctgaa gcttattgca   420
cgcatgatgg acggccaaag cgatcagttt ggccaacctg gtacgggcaa caacagtgcc   480
tcttccggta cttcttcatc tggcggttcc ccttttaacg atctatcagg ggggaaggcc   540
ccttccggca actcccсttc cggcaactac tctcccgtca gtaccttctc acccccatcc   600
acgccaacgt cccctacctc accgcttgat ttcccttctt ctcccaccaa agcagccggg   660
```

```
ggcagcacgc cggtaaccga tcatcctgac cctgttggta gcgcgggcat cggggccgga    720
aattcggtgg ccttcaccag cgccggcgct aatcagacgg tgctgcatga caccattacc    780
gtgaaagcgg gtcaggtgtt tgatggcaaa ggacaaacct tcaccgccgg ttcagaatta    840
ggcgatggcg gccagtctga aaaccagaaa ccgctgttta tactggaaga cggtgccagc    900
ctgaaaaacg tcaccatggg cgacgacggg gcggatggta ttcatcttta cggtgatgcc    960
aaaatagaca atctgcacgt caccaacgtg ggtgaggacg cgattaccgt taagccaaac   1020
agcgcgggca aaaaatccca cgttgaaatc actaacagtt ccttcgagca cgcctctgac   1080
aagatcctgc agctgaatgc cgatactaac ctgagcgttg acaacgtgaa ggccaaagac   1140
tttggtactt ttgtacgcac taacggcggt caacagggta actgggatct gaatctgagc   1200
catatcagcg cagaagacgg taagttctcg ttcgttaaaa gcgatagcga ggggctaaac   1260
gtcaatacca gtgatatctc actgggtgat gttgaaaacc actacaaagt gccgatgtcc   1320
gccaacctga aggtggctga atga                                          1344
```

The above nucleotide and amino acid sequences are disclosed and further described in U.S. Patent Application Serial No. 09/120,927 to Beer et al., which is hereby incorporated by reference in its entirety.

A hypersensitive response elicitor protein or polypeptide derived from *Pseudomonas syringae* has an amino acid sequence corresponding to SEQ. ID. No. 7 as follows:

```
Met Gln Ser Leu Ser Leu Asn Ser Ser Ser Leu Gln Thr Pro Ala Met
1               5                   10                  15

Ala Leu Val Leu Val Arg Pro Glu Ala Glu Thr Thr Gly Ser Thr Ser
            20                  25                  30

Ser Lys Ala Leu Gln Glu Val Val Val Lys Leu Ala Glu Glu Leu Met
        35                  40                  45

Arg Asn Gly Gln Leu Asp Asp Ser Ser Pro Leu Gly Lys Leu Leu Ala
    50                  55                  60

Lys Ser Met Ala Ala Asp Gly Lys Ala Gly Gly Gly Ile Glu Asp Val
65                  70                  75                  80

Ile Ala Ala Leu Asp Lys Leu Ile His Glu Lys Leu Gly Asp Asn Phe
                85                  90                  95

Gly Ala Ser Ala Asp Ser Ala Ser Gly Thr Gly Gln Gln Asp Leu Met
            100                 105                 110

Thr Gln Val Leu Asn Gly Leu Ala Lys Ser Met Leu Asp Asp Leu Leu
        115                 120                 125

Thr Lys Gln Asp Gly Gly Thr Ser Phe Ser Glu Asp Asp Met Pro Met
    130                 135                 140

Leu Asn Lys Ile Ala Gln Phe Met Asp Asp Asn Pro Ala Gln Phe Pro
145                 150                 155                 160

Lys Pro Asp Ser Gly Ser Trp Val Asn Glu Leu Lys Glu Asp Asn Phe
                165                 170                 175
```

```
Leu Asp Gly Asp Glu Thr Ala Ala Phe Arg Ser Ala Leu Asp Ile Ile
            180                 185                 190

Gly Gln Gln Leu Gly Asn Gln Gln Ser Asp Ala Gly Ser Leu Ala Gly
        195                 200                 205

Thr Gly Gly Gly Leu Gly Thr Pro Ser Ser Phe Ser Asn Asn Ser Ser
    210                 215                 220

Val Met Gly Asp Pro Leu Ile Asp Ala Asn Thr Gly Pro Gly Asp Ser
225                 230                 235                 240

Gly Asn Thr Arg Gly Glu Ala Gly Gln Leu Ile Gly Glu Leu Ile Asp
                245                 250                 255

Arg Gly Leu Gln Ser Val Leu Ala Gly Gly Gly Leu Gly Thr Pro Val
            260                 265                 270

Asn Thr Pro Gln Thr Gly Thr Ser Ala Asn Gly Gly Gln Ser Ala Gln
        275                 280                 285

Asp Leu Asp Gln Leu Leu Gly Gly Leu Leu Leu Lys Gly Leu Glu Ala
    290                 295                 300

Thr Leu Lys Asp Ala Gly Gln Thr Gly Thr Asp Val Gln Ser Ser Ala
305                 310                 315                 320

Ala Gln Ile Ala Thr Leu Leu Val Ser Thr Leu Leu Gln Gly Thr Arg
                325                 330                 335

Asn Gln Ala Ala Ala
            340
```

This hypersensitive response elicitor protein or polypeptide has a molecular weight of 34-35 kDa. It is rich in glycine (about 13.5%) and lacks cysteine and tyrosine. Further information about the hypersensitive response elicitor derived from *Pseudomonas syringae* is found in He, S. Y., et al., "*Pseudomonas syringae* pv. *syringae* Harpin$_{Pss}$: a Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," Cell 73:1255-1266 (1993), which is hereby incorporated by reference in its entirety. The DNA molecule encoding this hypersensitive response elicitor from *Pseudomonas syringae* has a nucleotide sequence corresponding to SEQ. ID. No. 8 as follows:

```
atgcagagtc tcagtcttaa cagcagctcg ctgcaaaccc cggcaatggc ccttgtcctg     60
gtacgtcctg aagccgagac gactggcagt acgtcgagca aggcgcttca ggaagttgtc    120
gtgaagctgg ccgaggaact gatgcgcaat ggtcaactcg acgacagctc gccattggga    180
aaactgttgg ccaagtcgat ggccgcagat ggcaaggcgg gcggcggtat tgaggatgtc    240
atcgctgcgc tggacaagct gatccatgaa aagctcggtg acaacttcgg cgcgtctgcg    300
gacagcgcct cgggtaccgg acagcaggac ctgatgactc aggtgctcaa tggcctggcc    360
aagtcgatgc tcgatgatct tctgaccaag caggatggcg ggacaagctt ctccgaagac    420
gatatgccga tgctgaacaa gatcgcgcag ttcatggatg acaatcccgc acagtttccc    480
```

```
aagccggact cgggctcctg ggtgaacgaa ctcaaggaag acaacttcct tgatggcgac   540
gaaacggctg cgttccgttc ggcactcgac atcattggcc agcaactggg taatcagcag   600
agtgacgctg gcagtctggc agggacgggt ggaggtctgg gcactccgag cagtttttcc   660
aacaactcgt ccgtgatggg tgatccgctg atcgacgcca ataccggtcc cggtgacagc   720
ggcaataccc gtggtgaagc ggggcaactg atcggcgagc ttatcgaccg tggcctgcaa   780
tcggtattgg ccggtggtgg actgggcaca cccgtaaaca ccccgcagac cggtacgtcg   840
gcgaatggcg gacagtccgc tcaggatctt gatcagttgc tgggcggctt gctgctcaag   900
ggcctggagg caacgctcaa ggatgccggg caaacaggca ccgacgtgca gtcgagcgct   960
gcgcaaatcg ccaccttgct ggtcagtacg ctgctgcaag gcacccgcaa tcaggctgca  1020
gcctga                                                             1026
```

The above nucleotide and amino acid sequences are disclosed and further described in U.S. Patent No. 5,708,139 to Collmer et al. and U.S. Patent No. 5,776,889 to Wei et al., which are hereby incorporated by reference in their entirety.

Another hypersensitive response elicitor protein or polypeptide derived from *Pseudomonas syringae* has an amino acid sequence corresponding to SEQ. ID. No. 9 as follows:

```
Met Ser Ile Gly Ile Thr Pro Arg Pro Gln Gln Thr Thr Thr Pro Leu
1               5                   10                  15

Asp Phe Ser Ala Leu Ser Gly Lys Ser Pro Gln Pro Asn Thr Phe Gly
            20                  25                  30

Glu Gln Asn Thr Gln Gln Ala Ile Asp Pro Ser Ala Leu Leu Phe Gly
        35                  40                  45

Ser Asp Thr Gln Lys Asp Val Asn Phe Gly Thr Pro Asp Ser Thr Val
    50                  55                  60

Gln Asn Pro Gln Asp Ala Ser Lys Pro Asn Asp Ser Gln Ser Asn Ile
65                  70                  75                  80

Ala Lys Leu Ile Ser Ala Leu Ile Met Ser Leu Leu Gln Met Leu Thr
                85                  90                  95

Asn Ser Asn Lys Lys Gln Asp Thr Asn Gln Glu Gln Pro Asp Ser Gln
            100                 105                 110

Ala Pro Phe Gln Asn Asn Gly Gly Leu Gly Thr Pro Ser Ala Asp Ser
        115                 120                 125

Gly Gly Gly Gly Thr Pro Asp Ala Thr Gly Gly Gly Gly Gly Asp Thr
    130                 135                 140

Pro Ser Ala Thr Gly Gly Gly Gly Gly Asp Thr Pro Thr Ala Thr Gly
145                 150                 155                 160

Gly Gly Gly Ser Gly Gly Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly
                165                 170                 175

Ser Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly Glu Gly Gly Val Thr
            180                 185                 190
```

```
Pro Gln Ile Thr Pro Gln Leu Ala Asn Pro Asn Arg Thr Ser Gly Thr
        195                 200                 205
Gly Ser Val Ser Asp Thr Ala Gly Ser Thr Glu Gln Ala Gly Lys Ile
    210                 215                 220
Asn Val Val Lys Asp Thr Ile Lys Val Gly Ala Gly Glu Val Phe Asp
225                 230                 235                 240
Gly His Gly Ala Thr Phe Thr Ala Asp Lys Ser Met Gly Asn Gly Asp
                245                 250                 255
Gln Gly Glu Asn Gln Lys Pro Met Phe Glu Leu Ala Glu Gly Ala Thr
            260                 265                 270
Leu Lys Asn Val Asn Leu Gly Glu Asn Glu Val Asp Gly Ile His Val
        275                 280                 285
Lys Ala Lys Asn Ala Gln Glu Val Thr Ile Asp Asn Val His Ala Gln
    290                 295                 300
Asn Val Gly Glu Asp Leu Ile Thr Val Lys Gly Glu Gly Gly Ala Ala
305                 310                 315                 320
Val Thr Asn Leu Asn Ile Lys Asn Ser Ser Ala Lys Gly Ala Asp Asp
                325                 330                 335
Lys Val Val Gln Leu Asn Ala Asn Thr His Leu Lys Ile Asp Asn Phe
            340                 345                 350
Lys Ala Asp Asp Phe Gly Thr Met Val Arg Thr Asn Gly Gly Lys Gln
        355                 360                 365
Phe Asp Asp Met Ser Ile Glu Leu Asn Gly Ile Glu Ala Asn His Gly
    370                 375                 380
Lys Phe Ala Leu Val Lys Ser Asp Ser Asp Asp Leu Lys Leu Ala Thr
385                 390                 395                 400
Gly Asn Ile Ala Met Thr Asp Val Lys His Ala Tyr Asp Lys Thr Gln
                405                 410                 415
Ala Ser Thr Gln His Thr Glu Leu
            420
```

This protein or polypeptide is acidic, glycine-rich, lacks cysteine, and is deficient in aromatic amino acids. The DNA molecule encoding this hypersensitive response elicitor from *Pseudomonas syringae* has a nucleotide sequence corresponding to SEQ. ID. No. 10 as follows:

```
tccacttcgc tgattttgaa attggcagat tcatagaaac gttcaggtgt ggaaatcagg    60
ctgagtgcgc agatttcgtt gataagggtg tggtactggt cattgttggt catttcaagg   120
cctctgagtg cggtgcggag caataccagt cttcctgctg gcgtgtgcac actgagtcgc   180
aggcataggc atttcagttc cttgcgttgg ttgggcatat aaaaaaagga acttttaaaa   240
acagtgcaat gagatgccgg caaaacggga accggtcgct gcgctttgcc actcacttcg   300
agcaagctca accccaaaca tccacatccc tatcgaacgg acagcgatac ggccacttgc   360
tctggtaaac cctggagctg gcgtcggtcc aattgcccac ttagcgaggt aacgcagcat   420
gagcatcggc atcacacccc ggccgcaaca gaccaccacg ccactcgatt tttcggcgct   480
```

```
aagcggcaag agtcctcaac caaacacgtt cggcgagcag aacactcagc aagcgatcga   540
cccgagtgca ctgttgttcg gcagcgacac acagaaagac gtcaacttcg gcacgcccga   600
cagcaccgtc cagaatccgc aggacgccag caagcccaac gacagccagt ccaacatcgc   660
taaattgatc agtgcattga tcatgtcgtt gctgcagatg ctcaccaact ccaataaaaa   720
gcaggacacc aatcaggaac agcctgatag ccaggctcct ttccagaaca acggcgggct   780
cggtacaccg tcggccgata gcgggggcgg cggtacaccg gatgcgacag gtggcggcgg   840
cggtgatacg ccaagcgcaa caggcggtgg cggcggtgat actccgaccg caacaggcgg   900
tggcggcagc ggtggcggcg gcacacccac tgcaacaggt ggcggcagcg gtggcacacc   960
cactgcaaca ggcggtggcg agggtggcgt aacaccgcaa atcactccgc agttggccaa  1020
ccctaaccgt acctcaggta ctggctcggt gtcggacacc gcaggttcta ccgagcaagc  1080
cggcaagatc aatgtggtga aagacaccat caaggtcggc gctggcgaag tctttgacgg  1140
ccacggcgca accttcactg ccgacaaatc tatgggtaac ggagaccagg gcgaaaatca  1200
gaagcccatg ttcgagctgg ctgaaggcgc tacgttgaag aatgtgaacc tgggtgagaa  1260
cgaggtcgat ggcatccacg tgaaagccaa aaacgctcag gaagtcacca ttgacaacgt  1320
gcatgcccag aacgtcggtg aagacctgat tacggtcaaa ggcgagggag gcgcagcggt  1380
cactaatctg aacatcaaga acagcagtgc caaaggtgca gacgacaagg ttgtccagct  1440
caacgccaac actcacttga aaatcgacaa cttcaaggcc gacgatttcg gcacgatggt  1500
tcgcaccaac ggtggcaagc agtttgatga catgagcatc gagctgaacg gcatcgaagc  1560
taaccacggc aagttcgccc tggtgaaaag cgacagtgac gatctgaagc tggcaacggg  1620
caacatcgcc atgaccgacg tcaaacacgc ctacgataaa acccaggcat cgacccaaca  1680
caccgagctt tgaatccaga caagtagctt gaaaaaaggg ggtggactc              1729
```

The above nucleotide and amino acid sequences are disclosed and further described in U.S. Patent Application Serial No. 09/120,817 to Collmer et al., which is hereby incorporated by reference in its entirety.

A hypersensitive response elicitor protein or polypeptide derived from *Pseudomonas solanacearum* has an amino acid sequence corresponding to SEQ. ID. No. 11 as follows:

```
Met Ser Val Gly Asn Ile Gln Ser Pro Ser Asn Leu Pro Gly Leu Gln
1               5                   10                  15

Asn Leu Asn Leu Asn Thr Asn Thr Asn Ser Gln Gln Ser Gly Gln Ser
            20                  25                  30

Val Gln Asp Leu Ile Lys Gln Val Glu Lys Asp Ile Leu Asn Ile Ile
        35                  40                  45
Ala Ala Leu Val Gln Lys Ala Ala Gln Ser Ala Gly Gly Asn Thr Gly
    50                  55                  60

Asn Thr Gly Asn Ala Pro Ala Lys Asp Gly Asn Ala Asn Ala Gly Ala
65                  70                  75                  80

Asn Asp Pro Ser Lys Asn Asp Pro Ser Lys Ser Gln Ala Pro Gln Ser
                85                  90                  95
```

```
Ala Asn Lys Thr Gly Asn Val Asp Asp Ala Asn Asn Gln Asp Pro Met
            100                 105                 110

Gln Ala Leu Met Gln Leu Leu Glu Asp Leu Val Lys Leu Leu Lys Ala
        115                 120                 125

Ala Leu His Met Gln Gln Pro Gly Gly Asn Asp Lys Gly Asn Gly Val
    130                 135                 140

Gly Gly Ala Asn Gly Ala Lys Gly Ala Gly Gly Gln Gly Gly Leu Ala
145                 150                 155                 160

Glu Ala Leu Gln Glu Ile Glu Gln Ile Leu Ala Gln Leu Gly Gly Gly
                165                 170                 175

Gly Ala Gly Ala Gly Gly Ala Gly Gly Gly Val Gly Gly Ala Gly Gly
            180                 185                 190

Ala Asp Gly Gly Ser Gly Ala Gly Gly Ala Gly Gly Ala Asn Gly Ala
        195                 200                 205

Asp Gly Gly Asn Gly Val Asn Gly Asn Gln Ala Asn Gly Pro Gln Asn
    210                 215                 220

Ala Gly Asp Val Asn Gly Ala Asn Gly Ala Asp Asp Gly Ser Glu Asp
225                 230                 235                 240

Gln Gly Gly Leu Thr Gly Val Leu Gln Lys Leu Met Lys Ile Leu Asn
                245                 250                 255

Ala Leu Val Gln Met Met Gln Gln Gly Gly Leu Gly Gly Gly Asn Gln
            260                 265                 270

Ala Gln Gly Gly Ser Lys Gly Ala Gly Asn Ala Ser Pro Ala Ser Gly
        275                 280                 285

Ala Asn Pro Gly Ala Asn Gln Pro Gly Ser Ala Asp Asp Gln Ser Ser
    290                 295                 300

Gly Gln Asn Asn Leu Gln Ser Gln Ile Met Asp Val Val Lys Glu Val
305                 310                 315                 320

Val Gln Ile Leu Gln Gln Met Leu Ala Ala Gln Asn Gly Gly Ser Gln
                325                 330                 335

Gln Ser Thr Ser Thr Gln Pro Met
            340
```

Further information regarding this hypersensitive response elicitor protein or polypeptide derived from *Pseudomonas solanacearum* is set forth in Arlat, M., et al., "PopA1, a Protein which Induces a Hypersensitive-like Response in Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-533 (1994), which is hereby incorporated by reference in its entirety. It is encoded by a DNA molecule from *Pseudomonas solanacearum* having a nucleotide sequence corresponding SEQ. ID. No. 12 as follows:

```
atgtcagtcg gaaacatcca gagcccgtcg aacctcccgg gtctgcagaa cctgaacctc    60
aacaccaaca ccaacagcca gcaatcgggc cagtccgtgc aagacctgat caagcaggtc   120
gagaaggaca tcctcaacat catcgcagcc ctcgtgcaga aggccgcaca gtcggcgggc   180
```

```
ggcaacaccg gtaacaccgg caacgcgccg gcgaaggacg gcaatgccaa cgcgggcgcc   240
aacgacccga gcaagaacga cccgagcaag agccaggctc cgcagtcggc caacaagacc   300
ggcaacgtcg acgacgccaa caaccaggat ccgatgcaag cgctgatgca gctgctggaa   360
gacctggtga agctgctgaa ggcggccctg cacatgcagc agcccggcgg caatgacaag   420
ggcaacggcg tgggcggtgc caacggcgcc aagggtgccg gcggccaggg cggcctggcc   480
gaagcgctgc aggagatcga gcagatcctc gcccagctcg gcggcggcgg tgctggcgcc   540
ggcggcgcgg gtggcggtgt cggcggtgct ggtggcgcgg atggcggctc cggtgcgggt   600
ggcgcaggcg gtgcgaacgg cgccgacggc ggcaatggcg tgaacggcaa ccaggcgaac   660
ggcccgcaga acgcaggcga tgtcaacggt gccaacggcg cggatgacgg cagcgaagac   720
cagggcggcc tcaccggcgt gctgcaaaag ctgatgaaga tcctgaacgc gctggtgcag   780
atgatgcagc aaggcggcct cggcggcggc aaccaggcgc agggcggctc gaagggtgcc   840
ggcaacgcct cgccggcttc cggcgcgaac ccgggcgcga accagcccgg ttcggcggat   900
gatcaatcgt ccggccagaa caatctgcaa tcccagatca tggatgtggt gaaggaggtc   960
gtccagatcc tgcagcagat gctggcggcg cagaacggcg gcagccagca gtccacctcg  1020
acgcagccga tgtaa                                                   1035
```

The above nucleotide and amino acid sequences are disclosed and further described in U.S. Patent No. 5,776,889 to Wei et al., which is hereby incorporated by reference in its entirety.

Other embodiments of the present invention include, but are not limited to, use of hypersensitive response elicitor proteins or polypeptides derived from *Erwinia carotovora* and *Erwinia stewartii*. Isolation of an *Erwinia carotovora* hypersensitive response elicitor protein or polypeptide is described in Cui, et al., "The RsmA Mutants of *Erwinia carotovora* subsp. *carotovora* Strain Ecc71 Overexpress *hrp*N_{Ecc} and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves," MPMI, 9(7):565-73 (1996), which is hereby incorporated by reference in its entirety. A hypersensitive response elicitor protein or polypeptide of *Erwinia stewartii* is set forth in Ahmad, et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," 8th Int'l. Cong. Molec. Plant-Microbe Interact., July 14-19, 1996 and Ahmad, et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," Ann. Mtg. Am. Phytopath. Soc., July 27-31, 1996, which are hereby incorporated by reference in their entirety.

Hypersensitive response elicitor proteins or polypeptides from various *Phytophthora* species are described in Kaman, et al., "Extracellular Protein Elicitors from Phytophthora: Most Specificity and Induction of Resistance to Bacterial and Fungal Phytopathogens," Molec. Plant-Microbe Interact., 6(1):15-25 (1993); Ricci, et

al., "Structure and Activity of Proteins from Pathogenic Fungi Phytophthora Eliciting Necrosis and Acquired Resistance in Tobacco," Eur. J. Biochem., 183:555-63 (1989); Ricci, et al., "Differential Production of Parasiticein, and Elicitor of Necrosis and Resistance in Tobacco, by Isolates of Phytophthora parasitica," Plant Path. 41:298-307 (1992); Baillreul, et al., "A New Elicitor of the Hypersensitive Response in Tobacco: A Fungal Glycoprotein Elicits Cell Death, Expression of Defense Genes, Production of Salicylic Acid, and Induction of Systemic Acquired Resistance," Plant J., 8(4):551-60 (1995), and Bonnet, et al., "Acquired Resistance Triggered by Elicitors in Tobacco and Other Plants," Eur. J. Plant Path., 102:181-92 (1996), which are hereby incorporated by reference in their entirety.

Another hypersensitive response elicitor protein or polypeptide which can be used in accordance with the present invention is derived from *Clavibacter michiganensis* subsp. *sepedonicus* and is described in U.S. Patent Application Serial No. 09/136,625, which is hereby incorporated by reference in its entirety.

Fragments of the above hypersensitive response elicitor proteins or polypeptides as well as fragments of full length elicitors from other pathogens can also be used according to the present invention.

Suitable fragments can be produced by several means. Subclones of the gene encoding a known elicitor protein can be produced using conventional molecular genetic manipulation for subcloning gene fragments, such as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York (1989), and Ausubel et al. (ed.), Current Protocols in Molecular Biology, John Wiley & Sons (New York, NY) (1999 and preceding editions), which are hereby incorporated by reference in their entirety. The subclones then are expressed *in vitro* or *in vivo* in bacterial cells to yield a smaller protein or polypeptide that can be tested for elicitor activity, e.g., using procedures set forth in Wei, Z-M., et al., Science 257: 85-88 (1992), which is hereby incorporated by reference in its entirety.

In another approach, based on knowledge of the primary structure of the protein, fragments of the elicitor protein gene may be synthesized using the PCR technique together with specific sets of primers chosen to represent particular portions of the protein. Erlich, H.A., et al., "Recent Advances in the Polymerase Chain Reaction," Science 252:1643-51 (1991), which is hereby incorporated by reference in

its entirety. These can then be cloned into an appropriate vector for expression of a truncated protein or polypeptide from bacterial cells as described above.

As an alternative, fragments of an elicitor protein can be produced by digestion of a full-length elicitor protein with proteolytic enzymes like chymotrypsin or *Staphylococcus* proteinase A, or trypsin. Different proteolytic enzymes are likely to cleave elicitor proteins at different sites based on the amino acid sequence of the elicitor protein. Some of the fragments that result from proteolysis may be active elicitors of resistance.

Chemical synthesis can also be used to make suitable fragments. Such a synthesis is carried out using known amino acid sequences for the elicitor being produced. Alternatively, subjecting a full length elicitor to high temperatures and pressures will produce fragments. These fragments can then be separated by conventional procedures (e.g., chromatography, SDS-PAGE).

An example of suitable fragments of a hypersensitive response elicitor which elicit a hypersensitive response are fragments of the *Erwinia amylovora* hypersensitive response elicitor protein or polypeptide of SEQ. ID. No. 3. The fragments can be a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 3, an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 3, or an internal fragment of the amino acid sequence of SEQ. ID. No. 3. The C-terminal fragment of the amino acid sequence of SEQ. ID. No. 3 can span amino acids 105 and 403 of SEQ. ID. No. 3. The N-terminal fragment of the amino acid sequence of SEQ. ID. No. 3 can span the following amino acids of SEQ. ID. No. 3: 1 and 98, 1 and 104, 1 and 122, 1 and 168, 1 and 218, 1 and 266, 1 and 342, 1 and 321, and 1 and 372. The internal fragment of the amino acid sequence of SEQ. ID. No. 3 can span the following amino acids of SEQ. ID. No. 3: 76 and 209, 105 and 209, 99 and 209, 137 and 204, 137 and 200, 109 and 204, 109 and 200, 137 and 180, and 105 and 180. DNA molecules encoding these fragments can also be utilized in the chimeric gene of the present invention.

Variants may also (or alternatively) be modified by, for example, the deletion or addition of amino acids that have minimal influence on the properties, secondary structure and hydropathic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the

protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification, or identification of the polypeptide.

The hypersensitive response elicitor proteins or polypeptides used in accordance with the present invention are preferably produced in purified form (preferably at least about 80%, more preferably 90%, pure) by conventional techniques. Typically, the protein or polypeptide of the present invention is secreted into the growth medium of recombinant host cells (discussed *infra*). Alternatively, the protein or polypeptide of the present invention is produced but not secreted into growth medium. In such cases, to isolate the protein, the host cell (e.g., *E. coli*) carrying a recombinant plasmid is propagated, lysed by sonication, heat, or chemical treatment, and the homogenate is centrifuged to remove bacterial debris. The supernatant is then subjected to sequential ammonium sulfate precipitation. The fraction containing the hypersensitive response elicitor protein or polypeptide of interest is subjected to gel filtration in an appropriately sized dextran or polyacrylamide column to separate the proteins. If necessary, the protein fraction may be further purified by HPLC.

Other hypersensitive response elicitors can be readily identified by isolating putative protein or polypeptide candidates and testing them for elicitor activity as described, for example, in Wei, Z-M., et al., "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992), which is hereby incorporated by reference in its entirety. Cell-free preparations from culture supernatants can be tested for elicitor activity (i.e., local necrosis) by using them to infiltrate appropriate plant tissues. Once identified, DNA molecules encoding a hypersensitive response elicitor can be isolated using standard techniques known to those skilled in the art.

DNA molecules encoding other hypersensitive response elicitor proteins or polypeptides can also be identified by determining whether such DNA molecules hybridizes under stringent conditions to a DNA molecule having the nucleotide sequence of SEQ. ID. Nos. 2, 4, 6, 8, 10, or 12. An example of suitable stringency conditions is when hybridization is carried out at a temperature of about 37°C using a hybridization medium that includes 0.9M sodium citrate ("SSC") buffer, followed by washing with 0.2x SSC buffer at 37°C. Higher stringency can readily be attained by increasing the temperature for either hybridization or washing conditions

or increasing the sodium concentration of the hybridization or wash medium. Nonspecific binding may also be controlled using any one of a number of known techniques such as, for example, blocking the membrane with protein-containing solutions, addition of heterologous RNA, DNA, and SDS to the hybridization buffer, and treatment with RNase. Wash conditions are typically performed at or below stringency. Exemplary high stringency conditions include carrying out hybridization at a temperature of about 42°C to about 65°C for up to about 20 hours in a hybridization medium containing 1M NaCl, 50 mM Tris-HCl, pH 7.4, 10 mM EDTA, 0.1% sodium dodecyl sulfate (SDS), 0.2% ficoll, 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin, and 50 μg/ml *E. coli* DNA, followed by washing carried out at between about 42°C to about 65°C in a 0.2x SSC buffer.

The DNA molecule encoding the hypersensitive response elicitor polypeptide or protein can be incorporated in cells using conventional recombinant DNA technology. Generally, this involves inserting the DNA molecule into an expression system to which the DNA molecule is heterologous (i.e. not normally present). The heterologous DNA molecule is inserted into the expression system or vector in proper sense orientation and correct reading frame. The vector contains the necessary elements for the transcription and translation of the inserted protein-coding sequences.

U.S. Patent No. 4,237,224 to Cohen and Boyer, which is hereby incorporated by reference in its entirety, describes the production of expression systems in the form of recombinant plasmids using restriction enzyme cleavage and ligation with DNA ligase. These recombinant plasmids are then introduced by means of transformation and replicated in unicellular cultures including prokaryotic organisms and eukaryotic cells grown in tissue culture.

Recombinant genes may also be introduced into viruses, such as vaccina virus. Recombinant viruses can be generated by transfection of plasmids into cells infected with virus.

Suitable vectors include, but are not limited to, the following viral vectors such as lambda vector system gt11, gt WES.tB, Charon 4, and plasmid vectors such as pBR322, pBR325, pACYC177, pACYC1084, pUC8, pUC9, pUC18, pUC19, pLG339, pR290, pKC37, pKC101, SV 40, pBluescript II SK +/- or KS +/- (see "Stratagene Cloning Systems" Catalog (1993) from Stratagene, La Jolla, Calif, which

is hereby incorporated by reference in its entirety), pQE, pIH821, pGEX, pET series (see F.W. Studier et. al., "Use of T7 RNA Polymerase to Direct Expression of Cloned Genes," Gene Expression Technology vol. 185 (1990), which is hereby incorporated by reference in its entirety), and any derivatives thereof. Recombinant molecules can be introduced into cells via transformation, particularly transduction, conjugation, mobilization, or electroporation. The DNA sequences are cloned into the vector using standard cloning procedures in the art, as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York (1989), which is hereby incorporated by reference in its entirety.

A variety of host-vector systems may be utilized to express the protein-encoding sequence(s). Primarily, the vector system must be compatible with the host cell used. Host-vector systems include but are not limited to the following: bacteria transformed with bacteriophage DNA, plasmid DNA, or cosmid DNA; microorganisms such as yeast containing yeast vectors; mammalian cell systems infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); and plant cells infected by bacteria. The expression elements of these vectors vary in their strength and specificities. Depending upon the host-vector system utilized, any one of a number of suitable transcription and translation elements can be used.

Different genetic signals and processing events control many levels of gene expression (e.g., DNA transcription and messenger RNA (mRNA) translation).

Transcription of DNA is dependent upon the presence of a promoter which is a DNA sequence that directs the binding of RNA polymerase and thereby promotes mRNA synthesis. The DNA sequences of eukaryotic promoters differ from those of prokaryotic promoters. Furthermore, eukaryotic promoters and accompanying genetic signals may not be recognized in or may not function in a prokaryotic system, and, further, prokaryotic promoters are not recognized and do not function in eukaryotic cells.

Similarly, translation of mRNA in prokaryotes depends upon the presence of the proper prokaryotic signals which differ from those of eukaryotes. Efficient translation of mRNA in prokaryotes requires a ribosome binding site called the Shine-Dalgarno ("SD") sequence on the mRNA. This sequence is a short nucleotide sequence of mRNA that is located before the start codon, usually AUG,

which encodes the amino-terminal methionine of the protein. The SD sequences are complementary to the 3'-end of the 16S rRNA (ribosomal RNA) and probably promote binding of mRNA to ribosomes by duplexing with the rRNA to allow correct positioning of the ribosome. For a review on maximizing gene expression, see Roberts and Lauer, Methods in Enzymology, 68:473 (1979), which is hereby incorporated by reference in its entirety.

Promoters vary in their "strength" (i.e. their ability to promote transcription). For the purposes of expressing a cloned gene, it is desirable to use strong promoters in order to obtain a high level of transcription and, hence, expression of the gene. Depending upon the host cell system utilized, any one of a number of suitable promoters may be used. For instance, when cloning in *E. coli*, its bacteriophages, or plasmids, promoters such as the T7 phage promoter, *lac* promoter, *trp* promoter, *rec*A promoter, ribosomal RNA promoter, the P_R and P_L promoters of coliphage lambda and others, including but not limited, to *lac*UV5, *omp*F, *bla*, *lpp*, and the like, may be used to direct high levels of transcription of adjacent DNA segments. Additionally, a hybrid *trp-lac*UV5 (*tac*) promoter or other *E. coli* promoters produced by recombinant DNA or other synthetic DNA techniques may be used to provide for transcription of the inserted gene.

Bacterial host cell strains and expression vectors may be chosen which inhibit the action of the promoter unless specifically induced. In certain operations, the addition of specific inducers is necessary for efficient transcription of the inserted DNA. For example, the *lac* operon is induced by the addition of lactose or IPTG (isopropylthio-beta-D-galactoside). A variety of other operons, such as *trp*, *pro*, etc., are under different controls.

Specific initiation signals are also required for efficient gene transcription and translation in prokaryotic cells. These transcription and translation initiation signals may vary in "strength" as measured by the quantity of gene specific messenger RNA and protein synthesized, respectively. The DNA expression vector, which contains a promoter, may also contain any combination of various "strong" transcription and/or translation initiation signals. For instance, efficient translation in *E. coli* requires an SD sequence about 7-9 bases 5' to the initiation codon ("ATG") to provide a ribosome binding site. Thus, any SD-ATG combination that can be utilized by host cell ribosomes may be employed. Such combinations include but are not

limited to the SD-ATG combination from the *cro* gene or the *N* gene of coliphage lambda, or from the *E. coli* tryptophan E, D, C, B or A genes. Additionally, any SD-ATG combination produced by recombinant DNA or other techniques involving incorporation of synthetic nucleotides may be used.

Once the isolated DNA molecule encoding the hypersensitive response elicitor polypeptide or protein has been cloned into an expression system, it is ready to be incorporated into a host cell. Such incorporation can be carried out by the various forms of transformation noted above, depending upon the vector/host cell system. Suitable host cells include, but are not limited to, bacteria, virus, yeast, mammalian cells, insect, plant, and the like.

Because it is desirable for recombinant host cells to secrete the hypersensitive response elicitor protein or polypeptide, it is preferable that the host cell also be transformed with a type III secretion system in accordance with Ham et al., "A Cloned *Erwinia chrysanthemi* Hrp (Type III Protein Secretion) System Functions in *Escherichia coli* to Deliver *Pseudomonas syringae* Avr Signals to Plant Cells and Secrete Avr Proteins in Culture," Microbiol. 95:10206-10211 (1998), which is hereby incorporated by reference in its entirety.

Isolation of the hypersensitive response elicitor protein or polypeptide from the host cell or growth medium can be carried out as described above.

The methods of the present invention can be performed by treating the fruit or vegetable either prior to or after harvest of the fruit or vegetable.

Suitable preharvest application methods include, without limitation, high or low pressure spraying of the entire plant and fruits. Suitable postharvest application methods include, without limitation, low or high pressure spraying, coating, or immersion. Other suitable application procedures (both preharvest and postharvest) can be envisioned by those skilled in the art provided they are able to effect contact of the hypersensitive response elicitor polypeptide or protein with the fruit or vegetable. Once treated, the fruits or vegetables can be handled, packed, shipped, and processed using conventional procedures to deliver the produce to processing plants or end-consumers.

The hypersensitive response elicitor polypeptide or protein can be applied to fruits or vegetables in accordance with the present invention alone or in a mixture with other materials. Alternatively, the hypersensitive response elicitor

polypeptide or protein can be applied separately to fruits or vegetables with other materials being applied at different times.

A composition suitable for treating fruits or vegetables in accordance with the application embodiment of the present invention contains an isolated hypersensitive response elicitor polypeptide or protein in a carrier. Suitable carriers include water, aqueous solutions, slurries, or dry powders. The composition preferably contains greater than about 500 nM hypersensitive response elicitor polypeptide or protein, although greater or lesser amounts of the hypersensitive response elicitor polypeptide or protein depending on the rate of composition application and efficacy of different hypersensitive response elicitor proteins or polypeptides.

Although not required, this composition may contain additional additives including fertilizer, insecticide, fungicide, nematacide, and mixtures thereof. Suitable fertilizers include $(NH_4)_2NO_3$. An example of a suitable insecticide is Malathion. Useful fungicides include Captan.

Other suitable additives include buffering agents, wetting agents, coating agents, and ripening agents. These materials can be used either to facilitate the process of the present invention or to provide additive benefits to inhibit postharvest disease and desiccation.

As indicated above, one embodiment of the present invention involves treating fruits or vegetables with an isolated hypersensitive response elicitor protein or polypeptide. The hypersensitive response elicitor protein or polypeptide can be isolated from its natural source (e.g., *Erwinia amylovora, Pseudomonas syringae*, etc.) or from recombinant source transformed with a DNA molecule encoding the protein or polypeptide.

Another aspect of the present invention relates to a DNA construct as well as host cells, expression systems, and transgenic plants which contain the heterologous DNA construct.

The DNA construct includes a DNA molecule encoding a hypersensitive response elicitor protein or polypeptide, a plant-expressible promoter operably coupled 5' to the DNA molecule and which is effective to transcribe the DNA molecule in fruit or vegetable tissue, and a 3' regulatory region operably coupled to the DNA molecule. Expression of the DNA molecule in fruit or vegetable

tissue imparts to a fruit or vegetable resistance against postharvest disease or desiccation.

Expression of such heterologous DNA molecules requires a suitable promoter which is operable in plant tissues. In some embodiments of the present invention, it may be desirable for the heterologous DNA molecule to be expressed in many, if not all, tissues. Such promoters yield constitutive expression of coding sequences under their regulatory control. Exemplary constitutive promoters include, without limitation, the nopaline synthase promoter (Fraley et al., Proc. Natl. Acad. Sci. USA 80:4803-4807 (1983), which is hereby incorporated by reference in its entirety) and the cauliflower mosaic virus 35S promoter (O'Dell et al., "Identification of DNA Sequences Required for Activity of the Cauliflower Mosaic Virus 35S Promoter," Nature, 313(6005):810-812 (1985), which is hereby incorporated by reference in its entirety).

While constitutive expression is generally suitable for expression of the DNA molecule, it should be apparent to those of skill in the art that temporally or tissue regulated expression may also be desirable, in which case any regulated promoter can be selected to achieve the desired expression. Typically, the temporally or tissue regulated promoters will be used in connection with the DNA molecule that are expressed at only certain stages of development or only in certain tissues.

In another embodiment of the present invention, expression of the heterologous DNA molecule is directed in a tissue-specific manner or environmentally-regulated manner (i.e., inducible promoters). Tissue-specific promoters under developmental control include promoters that initiate transcription only in certain tissues.

For example, the E4 and E8 promoters of tomato have been used to direct fruit-specific expression of a heterologous DNA sequence in transgenic tomato plants (Cordes et al., Plant Cell 1:1025-1034 (1989); Deikman et al., EMBO J. 7:3315-3320 (1988); and Della Penna et al., Proc. Natl. Acad. Sci. USA 83:6420-6424 (1986), which are hereby incorporated by reference in their entirety). Another fruit-specific promoter is the PG promoter (Bird et al., Plant Molec. Biol. 11:651-662 (1988), which is hereby incorporated by reference in its entirety). Another tissue-specific promoter is the AP2 promoter from the ovule-specific BEL1 gene promoter

described in Reiser et al., Cell 83:735-742 (1995), which is hereby incorporated by reference in its entirety.

Promoters useful for expression in seed tissues include, without limitation, the promoters from genes encoding seed storage proteins, such as napin, cruciferin, phaseolin, and the like (see U.S. Patent No. 5,420,034 to Kridl et al., which is hereby incorporated by reference in its entirety). Other suitable promoters include those from genes encoding embryonic storage proteins.

Promoters useful for expression in leaf tissue include the Rubisco small subunit promoter.

Promoters useful for expression in tubers, particularly potato tubers, include the patatin promoter.

Examples of environmental conditions that may affect transcription by inducible promoters include anaerobic conditions, elevated temperature, or the presence of light. In some plants, it may also be desirable to use promoters which are responsive to pathogen infiltration or stress. For example, it may be desirable to limit expression of the protein or polypeptide in response to infection by a particular pathogen of the plant. One example of a pathogen-inducible promoter is the *gst1* promoter from potato, which is described in U.S. Patent Nos. 5,750,874 and 5,723,760 to Strittmayer et al., which are hereby incorporated by reference in their entirety.

Expression of the DNA molecule in isolated plant cells or tissue or whole plants also utilizes appropriate transcription termination and polyadenylation of mRNA. Any 3' regulatory region suitable for use in plant cells or tissue can be operably linked to the first and second DNA molecules. A number of 3' regulatory regions are known to be operable in plants. Exemplary 3' regulatory regions include, without limitation, the nopaline synthase 3' regulatory region (Fraley, et al., "Expression of Bacterial Genes in Plant Cells," Proc. Nat'l. Acad. Sci. USA, 80:4803-4807 (1983), which is hereby incorporated by reference in its entirety) and the cauliflower mosaic virus 3' regulatory region (Odell, et al., "Identification of DNA Sequences Required for Activity of the Cauliflower Mosaic Virus 35S Promoter," Nature, 313(6005):810-812 (1985), which is hereby incorporated by reference in its entirety).

The promoter and a 3' regulatory region can readily be ligated to the DNA molecule using well known molecular cloning techniques described in Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Press, NY (1989), which is hereby incorporated by reference in its entirety.

One approach to transforming plant cells with a DNA molecule of the present invention is particle bombardment (also known as biolistic transformation) of the host cell. This can be accomplished in one of several ways. The first involves propelling inert or biologically active particles at cells. This technique is disclosed in U.S. Patent Nos. 4,945,050, 5,036,006, and 5,100,792, all to Sanford, et al., which are hereby incorporated by reference in their entirety. Generally, this procedure involves propelling inert or biologically active particles at the cells under conditions effective to penetrate the outer surface of the cell and to be incorporated within the interior thereof. When inert particles are utilized, the vector can be introduced into the cell by coating the particles with the vector containing the heterologous DNA. Alternatively, the target cell can be surrounded by the vector so that the vector is carried into the cell by the wake of the particle. Biologically active particles (e.g., dried bacterial cells containing the vector and heterologous DNA) can also be propelled into plant cells. Other variations of particle bombardment, now known or hereafter developed, can also be used.

Another method of introducing the DNA molecule into plant cells is fusion of protoplasts with other entities, either minicells, cells, lysosomes, or other fusible lipid-surfaced bodies that contain the DNA molecule. Fraley, et al., Proc. Natl. Acad. Sci. USA, 79:1859-63 (1982), which is hereby incorporated by reference in its entirety.

The DNA molecule may also be introduced into the plant cells by electroporation. Fromm, et al., Proc. Natl. Acad. Sci. USA, 82:5824 (1985), which is hereby incorporated by reference in its entirety. In this technique, plant protoplasts are electroporated in the presence of plasmids containing the DNA molecule. Electrical impulses of high field strength reversibly permeabilize biomembranes allowing the introduction of the plasmids. Electroporated plant protoplasts reform the cell wall, divide, and regenerate.

Another method of introducing the DNA molecule into plant cells is to infect a plant cell with *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes* previously transformed with the DNA molecule. Under appropriate conditions known in the art, the transformed plant cells are grown to form shoots or roots, and develop further into plants. Generally, this procedure involves inoculating the plant tissue with a suspension of bacteria and incubating the tissue for 48 to 72 hours on regeneration medium without antibiotics at 25-28°C.

Agrobacterium is a representative genus of the Gram-negative family Rhizobiaceae. Its species are responsible for crown gall (*A. tumefaciens*) and hairy root disease (*A. rhizogenes*). The plant cells in crown gall tumors and hairy roots are induced to produce amino acid derivatives known as opines, which are catabolized only by the bacteria. The bacterial genes responsible for expression of opines are a convenient source of control elements for chimeric expression cassettes. In addition, assaying for the presence of opines can be used to identify transformed tissue.

Heterologous genetic sequences such as a DNA molecule a hypersensitive response elicitor protein or polypeptide can be introduced into appropriate plant cells by means of the Ti plasmid of *A. tumefaciens* or the Ri plasmid of *A. rhizogenes*. The Ti or Ri plasmid is transmitted to plant cells on infection by *Agrobacterium* and is stably integrated into the plant genome. Schell, J., Science, 237:1176-83 (1987), which is hereby incorporated by reference in its entirety.

Plant tissue suitable for transformation include leaf tissue, root tissue, meristems, zygotic and somatic embryos, and anthers.

After transformation, the transformed plant cells can be selected and regenerated.

Preferably, transformed cells are first identified using, e.g., a selection marker simultaneously introduced into the host cells along with the DNA molecule of the present invention. Suitable selection markers include, without limitation, markers coding for antibiotic resistance, such as kanamycin resistance (Fraley, et al., Proc. Natl. Acad. Sci. USA, 80:4803-4807 (1983), which is hereby incorporated by reference in its entirety). A number of antibiotic-resistance markers are known in the art and other are continually being identified. Any known antibiotic-resistance marker can be used to transform and select transformed host cells in accordance with the present invention. Cells or tissues are grown on a selection media containing an

antibiotic, whereby generally only those transformants expressing the antibiotic resistance marker continue to grow.

Once a recombinant plant cell or tissue has been obtained, it is possible to regenerate a full-grown plant therefrom. Thus, another aspect of the present invention relates to a transgenic plant that includes a heterologous DNA molecule encoding a hypersensitive response elicitor protein or polypeptide, wherein the heterologous DNA molecule is under control or a promoter that induces transcription of the DNA molecule fruit or vegetable tissues. Preferably, the DNA molecule is stably inserted into the genome of the transgenic plant of the present invention.

Plant regeneration from cultured protoplasts is described in Evans, et al., Handbook of Plant Cell Cultures, Vol. 1: (MacMillan Publishing Co., New York, 1983); and Vasil I.R. (ed.), Cell Culture and Somatic Cell Genetics of Plants, Acad. Press, Orlando, Vol. I, 1984, and Vol. III (1986), which are hereby incorporated by reference in their entirety.

It is known that practically all plants can be regenerated from cultured cells or tissues, including both monocots and dicots.

Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts or a petri plate containing transformed explants is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, embryo formation can be induced in the callus tissue. These embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is usually reproducible and repeatable.

After the DNA molecule encoding the hypersensitive response elicitor protein or polypeptide is stably incorporated in transgenic plants, it can be transferred to other plants by sexual crossing or by preparing cultivars. With respect to sexual crossing, any of a number of standard breeding techniques can be used depending upon the species to be crossed. Cultivars can be propagated in accord with common agricultural procedures known to those in the field.

With regard to the use of the hypersensitive response elicitor protein or polypeptide in imparting postharvest disease resistance, absolute immunity against infection may not be conferred, but the severity of the disease can be reduced and symptom development can be delayed. Lesion number, lesion size, and extent of sporulation of fungal pathogens are all decreased. This method of controlling postharvest disease has the potential for controlling previously untreatable diseases and avoiding the use of infectious agents or environmentally harmful materials.

With respect to desiccation, complete protection against desiccation may not be conferred, but the severity of desiccation can be reduced. Desiccation protection inevitably will depend, at least to some extent, on other conditions such as storage temperatures, light exposure, etc. However, this method of controlling desiccation has the potential for eliminating some other treatments (i.e., use of coating waxes) which may contribute to reduced costs or, at least, substantially no increase in costs.

The methods of the present invention can be used to control a number of postharvest diseases caused by a variety of pathogens. These postharvest diseases and the causative agents which can be treated according to the present invention include, without limitation, the following: *Penicillium* (e.g., *Penicillium digitatum*), *Botrytis* (e.g., *Botrytis cinereaon*), *Phytophthora* (e.g., *Phytophthora citrophthora*), and *Erwinia* (e.g. *Erwinia carotovora*).

A further aspect of the present invention relates to a method of enhancing the longevity of fruit or vegetable ripeness.

According to one embodiment, this aspect of the present invention is carried out by treating a fruit or vegetable with a hypersensitive response elicitor protein or polypeptide under conditions effective to enhance the longevity of fruit or vegetable ripeness. Preferably, as noted above, the hypersensitive response elicitor protein or polypeptide is in isolated form. Treating of the fruit or vegetable can be performed either prior to harvest after harvest of the fruit or vegetable, using the techniques described above.

According to another embodiment, this aspect of the present invention is carried out by providing a transgenic plant or plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and then growing the transgenic plant or transgenic plant produced from the transgenic plant

seed under conditions effective to enhance the longevity of fruit or vegetable ripeness in a fruit or vegetable harvested from the transgenic plant. This aspect of the present invention may further include applying the hypersensitive response elicitor polypeptide or protein to the fruit or vegetable to enhance the longevity of fruit or vegetable ripeness. Treating of the fruit or vegetable can be performed either prior to harvest or after harvest of the fruit or vegetable, using the techniques described above.

The methods of the present invention can be utilized to treat a wide variety of fruits and vegetables to control postharvest disease or desiccation as well as enhance the longevity of fruit or vegetable ripeness. Fruits and vegetables which can be treated include any edible plant product, particularly those from traditional crop plants, such as seed, root, tuber, stem, leaf, flower, and fruit. Exemplary transgenic fruit plants and fruits that can be treated include, without limitation, apple, pear, peach, nectarine, apricot, plum, cherry, olive, melon, citrus, grape, strawberry, raspberry, blueberry, currant, pineapple, papaya, guava, banana, and kiwi. Exemplary transgenic vegetable plants and vegetables that can be treated include, without limitation, asparagus, potato, sweet potato, bean, pea, chicory, lettuce, parsley, basil, endive, cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, spinach, onion, garlic, eggplant, pepper, celery, leek, radish, carrot, squash, pumpkin, zucchini, cucumber, soybean, tobacco, tomato, sorghum, rhubarb, and sugarcane. Exemplary transgenic grain plants and grain products which can be treated include, without limitation, alfalfa, rice, wheat, barley, corn, and rye.

EXAMPLES

The following examples are provided to illustrate embodiments of the present invention but are by no means intended to limit its scope.

As used in the following Examples, Messenger® refers to a product available from Eden Bioscience Corporation (Bothell, Washington), which contains 3% by weight of harpin$_{Ea}$ as the active ingredient and 97% by weight inert ingredients. Harpin$_{Ea}$ is one type of hypersensitive response elicitor protein from *Erwinia amylovora*, identified herein by SEQ. ID. No. 3.

Example 1 – Effect of Treating Orange Fruits with Messenger® on Postharvest Orange Storage

On day 0, Fall-GLO orange fruits were treated by spraying Messenger® solution (ca. 15 ug/ml) or buffer solution (5mM KPO_4, pH 6.8) on the surface of fruits in a 22°C greenhouse. The Messenger® or buffer solutions on oranges were then dried by air, and the treated oranges were marked, mixed together, and put into a plastic container (Clear View 66 Qt/63L made by Sterilite Corporation). The container with treated oranges was then put into a 18°C growth chamber for storage. On day 7, orange fruits were inoculated with *Penicillium digitatum* and *Botrytis cinereaon* by spraying a 10^5 cfu/ml suspension on the surface of orange fruit. The above procedure was performed on 40 orange fruits per treatment.

Measurements of disease were conducted on days 20, 24, and 26 following treatment with Messenger® or buffer solution. Grades 0-5 indicate different disease scales -- Grade 0: No symptoms; Grade 1: 1/5 an individual fruit has disease symptoms; Grade 2: 2/5 an individual fruit has disease symptoms; Grade 3: 3/5 an individual fruit has disease symptoms; Grade 4: 4/5 an individual fruit has disease symptoms; Grade 5: whole fruit has disease symptoms. The results of these treatments are set forth in Table 1 below.

Table 1: Reduction of Disease Index in Oranges

Sample	Days After Treatment	Grade 0	1	2	3	4	5	Index	Efficacy	T-test p<0.05	p<0.01
Messenger®	20	33	3	1	0	2	1	0.09	58.14%	yes	yes
Buffer	20	23	8	0	2	6	1	0.22	n/a	-	-
Messenger®	24	25	2	6	4	1	2	0.20	45.21%	yes	yes
Buffer	24	16	7	3	3	4	7	0.37	n/a	-	-
Messenger®	26	19	4	6	4	5	2	0.29	36.96%	yes	yes
Buffer	26	16	3	3	0	7	11	0.46	n/a	-	-

The data listed in Table 1 above shows that the Messenger® was more effective than buffer as a fruit spray treatment in reducing disease index for *Penicillium digitatum* and *Botrytis cinereaon* and providing longer storage life. Messenger® treatment can reduce orange disease about 58.14% at 21 days, about 45.21% at 25 days, and 36.97% at 27 days after spraying treatment and 18°C storage conditions. T-test shows that there are statistically significant differences at both 95%

and 99% confidence levels for the results obtained from Messenger treatment® and buffer treatment.

Example 2 – **Effect of Treating Tomato (Hot House) Fruits with Messenger® on Postharvest Tomato Storage**

On day 0, Hot House tomato fruits were treated by spraying Messenger® solution (ca. 15 ug/ml) or buffer solution (5mM KPO_4, pH 6.8) on the surface of fruits in a 22°C greenhouse. The Messenger® or buffer solutions on tomatoes were then dried by air, and the treated tomatoes were marked, mixed together, and put into a plastic container (Clear View 66 Qt/63L made by Sterilite Corporation). The container with treated tomatoes was then put into 18°C growth chamber for storage. On day 7, tomatoes were inoculated with *Penicillium digitatum* and *Botrytis cinereaon* by spraying a 10^5 cfu/ml suspension on the surface of tomato fruit. The above procedure was performed on 15 tomatoes fruits per treatment.

Measurements of disease were conducted on days 21 and 27 following treatment with Messenger® or buffer solution. Grades are indicated according to the criteria set forth in Example 1. The results of these treatments are set forth in Table 2 below.

Table 2: Reduction of Disease Index in Tomatoes

Sample	Days After Treatment	Grade 0	1	2	3	4	5	Index	Efficacy	T-test $p<0.05$	$p<0.01$
Messenger®	21	7	2	2	3	1	0	0.25	58.70%	yes	yes
Buffer	21	3	1	2	1	2	6	0.61	n/a	-	-
Messenger®	27	2	2	4	3	2	2	0.49	30.19%	yes	yes
Buffer	27	1	1	2	2	3	6	0.71	n/a	-	-

The data listed in Table 2 above shows that the Messenger® was more effective than buffer as a fruit spray treatment in reducing disease index for *Penicillium digitatum* and *Botrytis cinereaon* and providing longer storage life. Messenger® treatment can reduce tomato disease about 58.70% at 21 days and about 30.19% at 27 days after spraying treatment and 18°C storage conditions. T-test shows that there are statistically significant differences at both 95% and 99% confidence levels for the results obtained from Messenger treatment® and buffer treatment.

Example 3 – Effect of Treating Grape Fruits with Messenger® on Postharvest Grape Storage

On day 0, Red G. Grape fruits were treated by spraying Messenger® solution (ca. 15 ug/ml) or buffer solution (5mM KPO_4, pH 6.8) on the surface of fruits in a 22°C greenhouse. The Messenger® or buffer solutions on grapes were then dried by air, and the treated grapes were marked, mixed together, and put into a plastic container (Clear View 66 Qt/63L made by Sterilite Corporation). The container with treated grapes was then put into a 18°C growth chamber for storage. On day 7, grapes were inoculated with *Penicillium digitatum* and *Botrytis cinereaon* by spraying a 10^5 cfu/ml suspension on the surface of grape fruit. The above procedure was performed on about 3700g of grape fruits per treatment.

Measurements of disease were conducted on days 14 and 21 following treatment with Messenger® or buffer solution. Grades are indicated according to the criteria set forth in Example 1. The results of these treatments are set forth in Table 3 below.

Table 3: Reduction of Disease Index in Grapes

Sample	Days After Treatment	Grade 0	1	2	3	4	5	Index	Efficacy	T-test p<0.05	p<0.01
Messenger®	14	225	99	42	39	21	13	0.20	45.65%	yes	yes
Buffer	14	98	130	91	52	38	48	0.38	n/a	-	-
Messenger®	21	66	83	126	98	39	27	0.42	39.35%	yes	yes
Buffer	21	18	36	64	72	119	137	0.69	n/a	-	-

The data listed in Table 3 above shows that the Messenger® was more effective than buffer as a fruit spray treatment in reducing disease index for *Penicillium digitatum* and *Botrytis cinereaon* and providing longer storage life. Messenger® treatment can reduce grape disease by about 45.65% at 14 days and about 39.35% at 21 days after spraying treatment and 18°C storage conditions. T-test shows that there are statistically significant differences at both 95% and 99% confidence levels for the results obtained from Messenger treatment® and buffer treatment.

<u>Example 4</u> – Effect of Treating Grapefruit Fruits with Messenger® on Postharvest Grapefruit Storage

On day 0, FL 33935 grapefruit fruits were treated by spraying Messenger® solution (ca. 15 ug/ml) or buffer solution (5mM KPO_4, pH 6.8) on the surface of fruits in a 22°C greenhouse. The Messenger® or buffer solutions on grapefruits were then dried by air, and the treated grapefruits were marked, mixed together, and put into a plastic container (Clear View 66 Qt/63L made by Sterilite Corporation). The container with treated grapefruit fruits was then put into a 18°C growth chamber for storage. On day 7, grapefruit fruits were inoculated with *Phytophthora citrophthora* by spraying a 10^5 cfu/ml suspension on the surface of grapefruit fruit. The above procedure was performed on 6 grapefruit fruits per treatment.

Measurements of disease were conducted on days 87, 97, 103, and 111 following treatment with Messenger® or buffer solution. Grades are indicated according to the criteria set forth in Example 1. The results of these treatments are set forth in Table 4 below.

Table 4: Reduction of Disease Index in Grapefruits

Sample	Days After Treatment	Grade 0	1	2	3	4	5	Index	Efficacy	T-test p<0.05	p<0.01
Messenger®	87	5	1	0	0	0	0	0.03	75.00%	yes	yes
Buffer	87	4	1	0	1	0	0	0.13	n/a	-	-
Messenger®	97	5	0	0	1	0	0	0.10	50.00%	yes	yes
Buffer	97	4	0	1	0	1	0	0.20	n/a	-	-
Messenger®	103	4	1	0	0	1	0	0.17	28.57%	yes	yes
Buffer	103	3	2	0	0	0	1	0.23	n/a	-	-
Messenger®	111	4	1	0	0	0	1	0.20	33.33%	yes	yes
Buffer	111	3	1	0	1	0	1	0.30	n/a	-	-

The data listed in Table 4 above shows that the Messenger® was more effective than buffer as a fruit spray treatment in reducing disease index for *Phytophthora citrophthora* and providing longer storage life. Messenger® treatment can reduce grapefruit disease by about 75.00% at 87 days, about 50.00% at 97 days, about 28.57% at 103 days, and about 33.33% at 111 days after spraying treatment and 18°C storage conditions. T-test shows that there are statistically significant

differences at both 95% and 99% confidence levels for the results obtained from Messenger treatment® and buffer treatment.

Example 5 - **Effect of Treating Apple (Fuji) Fruits with Messenger® on Postharvest Apple Storage**

On day 0, Fuji apple fruits were treated by spraying Messenger® solution (ca. 15 ug/ml) or buffer solution (5mM KPO_4, pH 6.8) on the surface of fruits in a 22°C greenhouse. The Messenger® or buffer solutions on apples were then dried by air, and the treated apples were marked, mixed together, and put into a plastic container (Clear View 66 Qt/63L made by Sterilite Corporation). The container with treated apples was then put into a 18°C growth chamber for storage. On day 7, apples were inoculated with *Penicillium digitatum* and *Phytophthora citrophora* by spraying a 10^5 cfu/ml suspension on the surface of apples. The above procedure was performed on 20 apples per treatment.

Measurements of disease were conducted on days 50, 61, 70, 78, and 85 following treatment with Messenger® or buffer solution. Grades are indicated according to the criteria set forth in Example 1. The results of these treatments are set forth in Table 5 below.

Table 5: Reduction of Disease Index in Apples

Sample	Days After Treatment	Grade 0	1	2	3	4	5	Index	Efficacy	T-test p<0.05	p<0.01
Messenger®	50	20	0	0	0	0	0	0.00	100.00%	yes	yes
Buffer	50	18	1	1	0	0	0	0.03	n/a	-	-
Messenger®	61	19	1	0	0	0	0	0.01	88.89%	yes	yes
Buffer	61	16	2	1	0	0	1	0.09	n/a	-	-
Messenger®	70	18	0	2	0	0	0	0.04	71.43%	yes	yes
Buffer	70	14	2	2	1	0	1	0.14	n/a	-	-
Messenger®	78	15	2	3	0	0	0	0.08	57.89%	yes	yes
Buffer	78	13	2	2	1	0	2	0.19	n/a	-	-
Messenger®	85	13	3	1	1	2	0	0.16	40.74%	yes	yes
Buffer	85	10	5	0	0	3	2	0.27	n/a	-	-

The data listed in Table 5 above shows that the Messenger® was more effective than buffer as a fruit spray treatment in reducing disease index for *Penicillium digitatum* and *Phytophthora citrophora* and providing longer storage life.

Messenger® treatment can reduce apple disease by about 100.00% at 51 days, 88.89% at 61 days, 71.43% at 70 days, 57.89% at 78 days, and 40.74% at 85 days after spraying treatment and 18°C storage conditions. T-test shows that there are statistically significant differences at both 95% and 99% confidence levels for the results obtained from Messenger treatment® and buffer treatment.

Example 6 - **Effect of Treating Apple (Granny Smith) Fruits with Messenger® on Postharvest Apple Storage**

On day 0, Granny Smith apple fruits were treated by spraying Messenger® solution (ca. 15 ug/ml) or buffer solution (5mM KPO_4, pH 6.8) on the surface of fruits in a 22°C greenhouse. The Messenger® or buffer solutions on apples were then dried by air, and the treated apples were marked, mixed together, and put into a plastic container (Clear View 66 Qt/63L made by Sterilite Corporation). The container with treated apples was then put into a 18°C growth chamber for storage. On day 7, apples were inoculated with *Penicillium digitatum* and *Phytophthora citrophora* by spraying a 10^5 cfu/ml suspension on the surface of apples. The above procedure was performed on 20 apples per treatment.

Measurements of disease were conducted on days 50, 61, 70, 78, and 85 following treatment with Messenger® or buffer solution. Grades are indicated according to the criteria set forth in Example 1. The results of these treatments are set forth in Table 6 below.

Table 6: Reduction of Disease Index in Apples

Sample	Days After Treatment	Grade 0	1	2	3	4	5	Index	Efficacy	T-test p<0.05	p<0.01
Messenger®	50	20	0	0	0	0	0	00.00	100.00%	yes	yes
Buffer	50	19	1	0	0	0	0	0.01	n/a	-	-
Messenger®	61	13	5	2	0	0	0	0.09	50.00%	yes	yes
Buffer	61	7	9	3	1	0	0	0.18	n/a	-	-
Messenger®	70	7	10	3	0	0	0	0.16	36.00%	yes	yes
Buffer	70	2	12	5	1	0	0	0.25	n/a	-	-
Messenger®	78	6	10	3	1	0	0	0.19	32.14%	yes	yes
Buffer	78	2	11	5	1	1	0	0.28	n/a	-	-
Messenger®	85	7	9	2	1	1	0	0.20	23.08	yes	yes
Buffer	85	4	10	4	1	0	1		n/a	-	-

The data listed in Table 6 above shows that the Messenger® was more effective than buffer as a fruit spray treatment in reducing disease index for *Penicillium digitatum* and *Phytophthora citrophora* and providing longer storage life. Messenger® treatment can reduce apple disease by about 100.00% at 51 days, 50.00% at 61 days, 36.00% at 70 days, 32.14% at 78 days, and 23.08% at 85 days after spraying treatment and 18°C storage conditions. T-test shows that there are statistically significant differences at both 95% and 99% confidence levels for the results obtained from Messenger treatment® and buffer treatment.

<u>Example 7</u> - Effect of Treating Tomato Fruits with Messenger® on Postharvest Tomato Storage

On day 0, tomato fruits were treated by spraying Messenger® solution (ca. 15 ug/ml) or buffer solution (5mM KPO_4, pH 6.8) on the surface of fruits in a 22°C greenhouse. After the Messenger® or buffer solutions on tomatoes were dried by air, the treated tomatoes were marked, mixed together, and put into a plastic container (Clear View 66 Qt/63L made by Sterilite Corporation). The container with treated tomatoes was then put into a 18°C growth chamber for storage. On day 7, tomatoes were inoculated with *Penicillium digitatum* and *Botrytis cinereaon* by spraying a 10^5 cfu/ml suspension on the surface of tomatoes. The above procedure was performed on 44 tomatoes per treatment.

Measurements of disease were conducted on days 18, 27, 35, and 42 following treatment with Messenger® or buffer solution. Grades are indicated according to the criteria set forth in Example 1. The results of these treatments are set forth in Table 7 below.

Table 7: Reduction of Disease Index in Tomatoes

Sample	Days After Treatment	Grade 0	1	2	3	4	5	Index	Efficacy	T-test p<0.05	p<0.01
Messenger®	18	21	18	5	0	0	0	0.13	37.78%	yes	yes
Buffer	18	11	21	12	0	0	0	0.20	n/a	-	-
Messenger®	27	16	18	9	1	0	0	0.18	25.00%	yes	yes
Buffer	27	8	24	8	4	0	0	0.24	n/a	-	-
Messenger®	35	7	14	13	10	0	0	0.32	16.67%	yes	yes
Buffer	35	1	16	15	10	2	0	0.38	n/a	-	-
Messenger®	42	1	10	9	12	9	3	0.52	12.88%	yes	yes
Buffer	42	0	3	15	10	11	5	0.60	n/a	-	-

The data listed in Table 7 above shows that the Messenger® was more effective than buffer as a fruit spray treatment in reducing disease index for *Penicillium digitatum* and *Botrytis cinereaon* and providing longer storage life. Messenger® treatment can reduce tomato disease by about 37.78% at 18 days, 25.00% at 27 days, 16.67% at 35 days, and 12.88% at 42 days after spraying treatment and 18°C storage conditions. T-test shows that there are statistically significant differences at both 95% and 99% confidence levels for the results obtained from Messenger® treatment and buffer treatment.

<u>Example 8</u> - Effect of Preharvest and Postharvest Messenger® Treatments on Tomato (Sanibel) Fruit Postharvest Storage

Plots of red and green Sanibel variety tomatoes were grown under either standard conditions or full Messenger® treatment over the course of the growing season. The standard conditions, also known as grower's standard, included fungicide treatment sprayed every seven days after transplanting using primarily fungicides containing copper-based active ingredients. The Messenger® treatment included six sprays at rate of 2.2 oz of the product per acre.

Red and green fruits were harvested from both the Messenger® treated and grower standard plots. It was noted that green tomatoes from the grower standard treatment plots were smaller (i.e. less mature) then green tomatoes from the messenger treated plants.

Harvested fruits were treated as follows:

(1) Fruits from Messenger® treated plots were further treated with Messenger® after harvest;

(2) Fruits from standard plots were treated with Messenger® after harvest;

(3) Fruits from Messenger® treated plots received no additional treatment following harvest; and

(4) Fruits from standard plots received no additional treatment following harvest.

Postharvest treatment of fruits from groups (1) and (2) was carried out by spraying with Messenger® at a rate of 20 ppm $harpin_{Ea}$ concentration using a

backpack-sprayer at about 30 p.s.i. The fruit were rolled during application to assure full coverage of the spray. The postharvest treated tomatoes were allowed to air dry and then tomatoes from groups (1)-(4) were marked and mixed together in storage in a single layer. Storage temperatures ranged from about 18 to 32°C and light intervals were approximately 12 hours of light and darkness. Tomatoes were checked daily for rot and desiccation for a total of 31 days after harvest. The results are shown in Table 8 below.

Table 8: Affect of Preharvest and Postharvest Treatment on Rot and Desiccation

Group	Ripe-ness	No. Fruit	Days After Harvest 14	19	21	22	23	25	31	No. Desiccated	% Marketable
(1) Pre/Postharvest Messenger®	Red	5	0	0	0	1	1	1	2	0	60%
	Green	4	0	0	0	0	0	0	0	0	100%
(2) Postharvest Messenger® Only	Red	5	0	0	0	0	0	0	0	4	20%
	Green	4	0	0	0	0	0	0	0	1	75%
(3) Preharvest Messenger® Only	Red	5	0	0	0	0	0	0	2	0	60%
	Green	5	0	0	0	0	0	0	0	0	100%
(4) No Messenger®	Red	5	1	3	1	5	5	5	5	0	0%
	Green	5	0	0	0	0	0	0	0	1	80%

The red tomatoes from group (4) all rotted by day 21. In contrast, all red tomatoes which received some form of Messenger® treatment showed reduced rate of decay and rot. Near the end of the trial a number of tomatoes were observed to have desiccated, exhibiting shriveled skins but no rot. These were included as non-marketable. These results are suggestive that both preharvest and postharvest Messenger® treatments can reduce the level of rotting and desiccation, thereby extending fresh storage time.

<u>Example 9</u> - Effect of Messenger on Post Harvested Maturity and Fruit Decay on Tomato During Ambient Storage

The tomatoes were grown under either standard conditions (identified in Example 8) or full Messenger® treatment over the course of the growing season (identified in Example 8) and then hand picked at the time of commercial harvest. Mature green fruit of uniform size (5/6) were collected throughout the field in four replicate samples of 25 fruit per sample, placed directly into fruit bags and transported to a laboratory facility for postharvest treatment and/or analysis. Three different treatment regimen were examined as follows:

(1) Fruits from Messenger® treated plots received no additional treatment following harvest;

(2) Fruits from standard plots were treated with Messenger® after harvest;

(3) Fruits from standard plots received no additional treatment following harvest.

Postharvest treatment of fruits from group (2) was carried out by dipping the fruit in a Messenger® solution (20 ppm harpin$_{Ea}$). The postharvest treated tomatoes were allowed to air dry and then tomatoes from groups (1)-(3) were marked and mixed together in tomato crates for storage. Storage temperatures ranged from about 23 to 26°C (75-80°F). The tomatoes were then rated for color development and decay over time using the rating scale below.

Grade	Description
1	Mature Green: When fruit cut in half, no seeds cut; fruit entirely green with no color break;
2	Pink: Initial sign of color break noticed on some areas of fruit; these areas are usually pink;
3	Pink/Red: Intermediate ripening: Fruit is not total red; some pink still remains;
4	Red: Fruit totally red in color;
5	Decay: Some areas of the fruit beginning to break down from decay.

The results of this test are summarized in Table 9 below.

Table 9: Affect of Preharvest and Postharvest Treatment on Maturity and Decay

Group	Days After Treatment	Grade 1	2	3	4	5	Index	Efficacy	T-test p<0.05	p<0.01
1	10	11	6	8	75	0	0.69	7.28%	yes	yes
2	10	5	7	11	77	0	0.72	3.81%	yes	yes
3	10	5	3	6	86	1	0.75	N/A	N/A	N/A
1	14	4	5	5	86	0	0.75	2.61%	yes	yes
2	14	2	6	5	87	0	0.75	1.57%	yes	yes
3	14	2	4	4	89	1	0.77	N/A	N/A	N/A
1	17	0	0	3	92	5	0.80	3.37%	yes	yes
2	17	0	1	4	82	13	0.81	2.16%	yes	yes
3	17	0	0	1	82	17	0.83	N/A	N/A	N/A
1	20	0	0	0	89	11	0.82	2.61%	yes	yes
2	20	0	0	0	80	20	0.84	0.47%	yes	yes
3	20	0	0	1	76	23	0.84	N/A	N/A	N/A

The data generated in this trial indicate that treatment of tomatoes with Messenger®, either through field sprays or as a post harvest dip, resulted in earlier fruit red ripening compared to grower's standard. In addition, although early ripening was observed, the Messenger® treatments maintained the red ripe condition longer than the grower's standard with delay of breakdown and decay.

<u>Example 10</u> - Effect on Messenger on Post Harvested Maturity and Fruit Decay of Tomato Under Cold Storage Conditions

The tomatoes were grown under either standard conditions (identified in Example 8) or full Messenger® treatment over the course of the growing season (identified in Example 8) and then hand picked at the time of commercial harvest. Mature green fruit of uniform size (5/6) were collected throughout the field in four replicate samples of 25 fruit per sample, placed directly into fruit bags and transported to a laboratory facility for postharvest treatment and/or analysis. Four different treatment regimen were examined as follows:

(1) Fruits from Messenger® treated plots received no additional treatment following harvest;

(2) Fruits from Messenger® treated plots were further treated with Messenger® after harvest;

(3) Fruits from standard plots were treated with Messenger® after harvest; and

(4) Fruits from standard plots received no additional treatment following harvest.

Postharvest treatment of fruits from groups (2) and (3) were carried out by dipping the fruit in a Messenger® solution (20 ppm harpin$_{Ea}$). The postharvest treated tomatoes were allowed to air dry and then tomatoes from groups (1)-(4) were marked and mixed together in tomato crates for storage in a Custom Packing House cooler at 11°C (52°F). The tomatoes were then rated for color development and decay over time using the rating scale described in Example 8. The results of this study appear in Table 10 below.

Table 10: Affect of Preharvest and Postharvest Treatment on Maturity and Decay

Group	Days After Treatment	Grade 1	2	3	4	5	Index	Efficacy	T-test p<0.05	p<0.01
1	7	66	34	0	0	0	0.27	0.00%	yes	yes
2	7	67	33	0	0	0	0.27	0.75%	yes	yes
3	7	76	24	0	0	0	0.27	7.46%	yes	yes
4	7	68	30	2	0	0	0.27	N/A	yes	yes
1	10	59	31	8	0	0	0.30	7.53%	yes	yes
2	10	60	28	12	0	0	0.30	5.00%	yes	yes
3	10	65	35	0	0	0	0.27	15.63%	yes	yes
4	10	49	42	9	0	0	0.32	N/A	N/A	N/A
1	17	19	35	28	18	0	0.49	7.20%	yes	yes
2	17	20	38	28	14	0	0.47	10.61%	yes	yes
3	17	19	28	39	14	0	0.50	6.06%	yes	yes
4	17	17	27	31	25	0	0.53	N/A	N/A	N/A
1	21	11	28	29	32	0	0.56	6.62%	N/A	N/A
2	21	15	26	37	22	0	0.53	11.92%	yes	yes
3	21	10	33	35	22	0	0.54	10.93%	yes	yes
4	21	10	18	32	40	0	0.60	N/A	N/A	N/A
1	26	3	15	23	59	0	0.68	-2.26%	yes	yes
2	26	9	19	25	41	6	0.63	4.39%	yes	yes
3	26	3	23	31	43	0	0.63	5.00%	yes	yes
4	26	2	19	23	50	1	0.66	N/A	N/A	N/A
1	32	3	15	23	59	0	0.68	-2.26%	yes	yes
2	32	9	19	25	41	6	0.63	4.39%	yes	yes
3	32	3	23	31	43	0	0.63	5.00%	yes	yes
4	32	2	19	23	50	1	0.66	N/A	N/A	N/A

Table 10 cont.

Group	Days After Treatment	Grade 1	2	3	4	5	Index	Efficacy	T-test p<0.05	p<0.01
1	38	0	4	10	84	2	0.77	0.26%	yes	yes
2	38	1	10	15	65	9	0.74	3.64%	yes	yes
3	38	1	5	14	78	2	0.75	2.60%	yes	yes
4	38	0	3	13	80	4	0.77	N/A	N/A	N/A
1	45	0	3	11	74	12	0.79	2.95%	yes	yes
2	45	1	4	12	69	14	0.78	3.93%	yes	yes
3	45	0	1	11	81	7	0.79	3.19%	yes	yes
4	45	0	0	10	73	17	0.81	N/A	N/A	N/A
1	50	0	3	10	63	23	0.82	3.55%	yes	yes
2	50	0	4	11	58	27	0.82	3.55%	yes	yes
3	50	0	0	8	78	14	0.81	4.02%	yes	yes
4	50	0	0	3	71	26	0.85	N/A	N/A	N/A
1	55	0	0	0	73	27	0.85	1.84%	yes	yes
2	55	0	0	0	68	32	0.86	0.69%	yes	yes
3	55	0	0	2	80	18	0.83	4.37%	yes	yes
4	55	0	0	0	65	35	0.87	N/A	N/A	N/A
1	60	0	0	0	65	35	0.87	2.47%	yes	yes
2	60	0	0	0	63	37	0.87	2.02%	yes	yes
3	60	0	0	0	74	26	0.85	4.48%	yes	yes
4	60	0	0	0	54	46	0.89	N/A	N/A	N/A
1	65	0	0	0	53	47	0.89	1.76%	yes	yes
2	65	0	0	0	58	42	0.88	2.86%	yes	yes
3	65	0	0	0	65	35	0.87	4.40%	yes	yes
4	65	0	0	0	45	55	0.91	N/A	N/A	N/A

In previous trials when tomatoes were treated with Messenger® in the field and/or with a post harvest dip, the fruit appeared to develop to red ripe more quickly than the grower's standard, when held at ambient temperatures (75-80°F). Although this early ripening was observed, these red fruit did not begin to decay earlier than the grower's standard. In this study, the fruit were held at a constant 52°F in a commercial cold storage room at a tomato packinghouse facility. It appears that this lower temperature slows the ripening process, as would be expected, and Messenger® treatments did not increase the rate of the red ripening for the first 30 days, as observed in previous tests. The Messenger® treatments did, however,

seem to maintain the red ripe condition longer than the grower's standard without breakdown and decay.

<u>Example 11</u> - Effect of Messenger on Post Harvested Maturity and Fruit Decay on Tomato

The tomatoes were grown under either standard conditions (identified in Example 8) or full Messenger® treatment over the course of the growing season (identified in Example 8) and then hand picked at the time of commercial harvest. Mature green fruit of uniform size (5/6) were collected throughout the field in four replicate samples of 25 fruit per sample, placed directly into fruit bags and transported to a laboratory facility for postharvest treatment and/or analysis. Four different treatment regimen were examined as follows:

(1) Fruits from Messenger® treated plots received no additional treatment following harvest;

(2) Fruits from Messenger® treated plots were further treated with Messenger® after harvest;

(3) Fruits from standard plots were treated with Messenger® after harvest; and

(4) Fruits from standard plots received no additional treatment following harvest.

Postharvest treatment of fruits from groups (2) and (3) were carried out by dipping the fruit in a Messenger® solution (20 ppm $\text{harpin}_{\text{Ea}}$). The postharvest treated tomatoes were allowed to air dry and then tomatoes from groups (1)-(4) were marked and mixed together in tomato crates for storage. Storage temperatures ranged from about 23 to 26°C (75-80°F). The tomatoes were then rated for color development and decay over time using the commercial rating scale from the Florida Tomato Committee color guide as follows:

<u>Grade</u>	<u>Description</u>
1	Green: When fruit cut in half, no seeds cut; fruit entirely green with no color break;
2	Breakers: Initial sign of color break on 10% or less of the area of fruit; these areas are usually pink;
3	Turning: Pink or red on 10 to 30% of the fruit surface;

4 Pink: Pink or red on 30 to 60% of the fruit surface;

5 Light Red: Pink on over 60% of fruit surface and red color no more than 90% of fruit surface;

6 Red: Fruit totally red in color; and

7 Decay: Some areas of the fruit beginning to break down from decay.

The results of this treatment are set forth in Table 11 below.

Table 11: Affect of Preharvest and Postharvest Treatment on Maturity and Decay Data

Group	Days After Treatment	Grade 1	2	3	4	5	6	7	Index	Efficacy	T-test p<0.05	p<0.01
1	3	80	18	2	0	0	0	0	0.17	0.00%	no	no
2	3	73	17	9	1	0	0	0	0.20	-13.11%	yes	yes
3	3	78	19	3	0	0	0	0	0.18	-2.46%	yes	yes
4	3	80	18	2	0	0	0	0	0.17	N/A	no	no
1	7	36	23	22	12	5	2	0	0.33	3.72%	yes	no
2	7	37	23	17	19	4	0	0	0.33	4.96%	yes	no
3	7	40	17	15	18	9	1	0	0.35	0.00%	yes	no
4	7	35	22	19	15	8	1	0	0.35	N/A	no	no
1	14	2	5	8	8	13	65	0	0.74	8.02%	yes	yes
2	14	2	3	5	9	8	72	1	0.77	4.44%	yes	yes
3	14	4	4	7	8	17	60	0	0.73	9.41%	yes	yes
4	14	0	0	6	5	13	72	4	0.80	N/A	no	no
1	17	0	0	2	3	6	89	0	0.83	2.51%	yes	yes
2	17	1	1	1	0	7	88	2	0.83	2.35%	yes	yes
3	17	1	2	0	0	9	88	0	0.83	3.18%	yes	yes
4	17	0	0	0	0	7	89	4	0.85	N/A	no	no
1	21	0	0	0	0	0	97	3	0.86	1.31%	yes	yes
2	21	0	0	0	0	0	97	3	10.86	1.31%	yes	yes
3	21	0	0	0	0	3	95	2	0.86	1.96%	yes	yes
4	21	0	0	0	0	1	87	12	0.87	N/A	no	no
1	28	0	0	0	0	0	85	15	0.88	2.84%	yes	yes
2	28	0	0	0	0	0	91	9	0.87	3.79%	yes	yes
3	28	0	0	0	0	0	81	19	0.88	2.21%	yes	yes
4	28	0	0	0	0	0	67	33	0.90	N/A	no	no
1	32	0	0	0	0	0	22	78	0.97	2.16%	yes	yes
2	32	0	0	0	0	0	16	84	0.98	1.30%	yes	yes
3	32	0	0	0	0	0	55	45	0.92	6.93%	yes	yes
4	32	0	0	0	0	0	7	93	0.99	N/A	no	no

Table 11 Cont.

Group	Days After Treatment	Grade 1	2	3	4	5	6	7	Index	Efficacy	T-test p<0.05	p<0.01
1	37	0	0	0	0	0	14	86	0.98	1.15%	yes	yes
2	37	0	0	0	0	0	7	93	0.00	0.14%	yes	yes
3	37	0	0	0	0	0	9	91	0.99	0.43%	yes	yes
4	37	0	0	0	0	0	6	94	0.99	N/A	no	no
1	42	0	0	0	0	0	12	88	0.98	1.01%	yes	yes
2	42	0	0	0	0	0	7	93	0.99	0.29%	yes	yes
3	42	0	0	0	0	0	8	92	0.99	0.43%	yes	yes
4	42	0	0	0	0	0	5	95	0.99	N/A	no	no
1	45	0	0	0	0	0	8	92	0.99	0.57%	no	no
2	45	0	0	0	0	0	4	96	0.99	0.00%	no	no
3	45	0	0	0	0	0	4	96	0.99	0.00%	no	no
4	45	0	0	0	0	0	4	96	0.99	N/A	no	no
1	50	0	0	0	0	0	7	93	0.99	0.43%	no	no
2	50	0	0	0	0	0	4	96	0.99	0.00%	no	no
3	50	0	0	0	0	0	4	96	0.99	0.00%	no	no
4	50	0	0	0	0	0	4	96	0.99	N/A	no	no

In previous trials tomatoes treated with Messenger® in the field and/or with a post harvest dip appeared to develop to red ripe more quickly, but decayed slower than the grower's standard. The data generated from this trial support these observations. By twenty-one days post harvest, 97% of the Messenger® treated tomatoes were full red ripe, compared to 87% of the grower's standard. Although it may be assumed that fruit which reach maturity more quickly will also start to break down more quickly, the results of the present Examples surprisingly demonstrate that these earlier-maturing tomatoes were actually 15% slower to decay than the grower's standard tomatoes. This phenomenon should be of great interest of several segments of the tomato market. The growers may be able to reduce ethylene gashouse timings, and the retail market should be able to significantly reduce inventory shrinkage.

Although the invention has been described in detail for the purpose of illustration, it is understood that such detail is solely for that purpose, and variations can be made therein by those skilled in the art without departing from the spirit and scope of the invention which is defined by the following claims.